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A SIMPLIFIED METHOD FOR THE DETERMINATION OF CAROTENE IN FLOUR EXTRACTS [1]

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The importance of flour colour to the milling industry has led to the development of numerous methods for its evaluation. The colour of flour as viewed by the eye is the sum of a number of factors which may be divided into two broad groups: (1) Moisture, granulation, vitreousness, bran particles, and foreign matter; and (2) carotenoid pigments.

Attempts to evaluate flour colour directly either in the dry state or by the well known Pekar test, while valuable in mill control, have not until recently been subjected to quantitative expression. The standards hitherto necessary to the success of the methods commonly used are not of a permanent nature and so many other factors enter into these estimations that the results obtained are purely relative. These difficulties have in part been met by Hardy, Cole, and Ricker (1929) who employed an automatically recording spectrophotometer for determining the spectral distribution curves of a number of commercial flours, and presented a simplified method of expressing the results. An instrument of this type eliminates colour standards, but the high cost of the equipment is an obstacle to its wide adoption. More recently Ruter (1931) has applied the Pulfrich photometer to the quantitative measurement of the colour of wet and dry flour slicks but with only partial success.

Methods of evaluation based on a determination of the carotenoid content have met with greater success and possess certain distinct advantages over those which attempt to measure flour colour directly. From

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the mill chemist's standpoint, control of the factors comprising group (1) is largely a matter of suitable mechanical treatment in the milling process, whereas the carotenoid pigments comprising group (2) are inherent in the wheat and can only be controlled by suitable bleaching methods. In wheat breeding, the carotenoid content is an important factor in the selection of new varieties.

The simplest and most familiar method of estimating the carotenoid content of wheat and flour is the so-called "gasoline colour value" devised by Winton (1911) and adopted without substantial modification in the "Methods for the Analysis of Cereals and Cereal Products" of the A. A. C. C. (1928). Briefly, this test consists of a 16-hour extraction of a 20-gram sample with 100 cc. of colourless gasoline, filtration to remove suspended flour particles, and a comparison of the clear filtrate with 0.005% potassium chromate solution in a suitable colorimeter, or with a series of potassium chromate standards in Nessler tubes. Unfortunately, while simple in principle, this test is decidedly deficient in several respects. This is evidenced by the volume of literature dealing with modifications, none of which appear to have met with general favour.

A questionnaire sent to several cereal chemists by Coleman (1930) revealed that the test is rarely used in a practical way by the large mills, which led him to report unfavourably to the A. O. A. C. concerning the advisability of collaborative work on the gasoline colour value method. Furthermore, he points out that a more precise method of determining carotenoid pigments has been worked out by Ferrari and Bailey (1929 and 1929a) based on the studies of Schertz (1923) who developed the use of a visual spectrophotometer for the determination of carotene.

The spectrophotometric method gives an accurate quantitative measure of pigment concentration but is not well adapted to routine use. The instrument is costly and great care is required in obtaining an extract free from turbidity. Whiteside (1931) considered it advisable to test his extracts in an ultra-microscope. Furthermore, the measurement of transmittancy at the wave length employed (4358 Ångström units) is rather tedious. It must be regarded essentially as a research method and its general adoption in routine work seems unlikely. It would therefore appear that improvements in the Winton method or the development of some simpler procedure for determining carotene would be highly desirable.

The literature on colorimetric methods of estimating flour pigmentation by means of the gasoline colour test has been recently reviewed by Visser't-Hooft and DeLeeuw (1928) and Ferrari and Bailey (1929 and 1929a) and need not be detailed here. The principal difficulty appears to reside in the selection of a standard solution of suitable colour

with which to compare the gasoline extract. Pure carotene, on account of its instability, is generally considered to be unsuitable for use as a standard, and a number of other substances have therefore been employed. Willstätter and Stoll (1913), in their studies on pure carotene, used potassium dichromate and also alizarin dissolved in chloroform. Winton (1911) employed potassium chromate; Jørgensen (1927) modified Winton's standard by dissolving the potassium chromate in buffer solutions of suitable pH. Kent-Jones and Herd (1927) recommended a mixture of potassium chromate and cobalt nitrate; Sprague (1928) in studies on carotene employed an aqueous solution of naphthol yellow and orange G, while Kuhn and Brockmann (1932) used azobenzene.

It is quite obvious from the number of standards suggested that considerable difficulty is experienced in obtaining a solution that matches the colour of carotene or of flour extract. In the instance of flour this is further complicated by variations in the colour of gasoline extracts of different samples. This was pointed out by Winton (1911) who states that "usually the gasoline solution is a true yellow, but sometimes, especially in the case of clear flour, a slight brownish tint is discernible, although this is not sufficient to seriously interfere with the accuracy of the test." In the authors' experience with experimentally milled flours this brownish colour is met quite frequently and is fatal to accurate matching with a potassium chromate standard by the conventional method.

Aside from these inherent colour differences the problem of precise matching is further complicated because the yellow region of the spectrum is probably the most difficult in which to obtain colorimetric balance. This fact does not appear to be generally recognized, but is indicated by Yoe (1928), who advises that in developing new colorimetric methods, reactions that produce red, green, or blue solutions should be used. It probably explains the observations of Schertz (1923) who found that three observers, using the same carotene solution in both sides of a Duboscq colorimeter, obtained results varying by approximately 40%. This fundamental difficulty coupled with the unsatisfactory colour of the standard and variations in the hue of different flour extracts makes accurate matching a matter of considerable difficulty. It is only by constant practise, and a mental disregard of hue differences that even approximately relative results can be obtained.

Larmour and Brockinton,[5] in a statistical study of some of the sources of error in the estimation of gasoline colour value of experimentally milled flours, found that variations in solvent, depths of colorimeter setting and replications made on different days each show signifi-

[5] Unpublished data. Personal communication from Dr. R. K. Larmour, University of Saskatchewan, Saskatoon, Sask.

cant variance. If these three factors were kept constant, a difference of 0.10 units of gasoline colour between the means of duplicate determinations was found to be significant. On the other hand, if the values were obtained on different days with uniform solvent and colorimeter settings, a difference of 0.22 units represented the minimum significant difference between samples. With variations in all three factors this value must be increased to 0.42 units. It would appear from the foregoing that the gasoline colour test, as ordinarily applied, lacks sufficient accuracy to serve as an indication of other than relatively wide differences in carotenoid content.

In the investigations reported in the literature, no application has been made of the fact that the colour of a body is dependent upon the composition of the incident light. Carotene solutions appear yellow in white light because they absorb most of the green and blue radiation, transmitting substantially only yellow and red. Certain alterations in the spectral character of the incident radiation will consequently produce changes in the colour of the body. The colour difference between potassium chromate solution and a flour extract viewed with white light is due to their differential absorption of the various component wave lengths. If, however, these solutions are viewed by radiation of a narrow region of the spectrum at which their spectral distribution curves are similar or identical, the colour of the two solutions will be approximately the same. In other words, it is immaterial what the colours are in white light; as long as the spectral characteristics approximately coincide in some region of the spectrum the colours when viewed with radiation from that region will match, the chief difference discernible being one of intensity.

This principle has been little applied in colorimetry, probably because the majority of such determinations use a standard that is identical in chemical composition and therefore in spectral characteristics with the unknown. There are many cases, however, where permanent standards are required which by reason of different chemical composition are not spectrally identical with the substance determined. Under these circumstances this principle should be capable of general application.

Problem

Some years ago it was observed in this laboratory that the difference in hue between flour extracts and potassium chromate solution, so evident in white light, was greatly minimized, if not totally eliminated, when viewed by the radiation of the mercury arc, thus greatly facilitating the obtaining of a colorimetric balance. The average of a series of comparative readings using daylight and mercury arc radiation gave values by the latter amounting to only 51.5% of the former. The use

of the mercury arc thus cuts the usual values approximately in half. Evidence was obtained that the reduced spread in gasoline colour values between flours was more than offset by the gain in accuracy of matching and the method was therefore employed in routine tests in this laboratory, relative values only being required.

It was thought that the mercury arc results might be more highly correlated with the carotenoid content of the flours than the usual values but no facilities were available to investigate this point. The recent acquisition of a Bausch and Lomb Universal Spectrophotometer provided a means for the accurate determination of carotenoid content and also for an investigation of the comparative spectral characteristics of flour extracts and the colorimetric standards commonly employed. The present study was undertaken with the object of investigating these points with a view to the development of a simplified method for the quantitative determination of carotenoid pigmentation in wheat flours.

Experimental

Materials and Methods

The experimental material employed in these studies consisted for the most part of bleached and unbleached flours experimentally milled from a series of new wheat varieties submitted by the plant breeders of Western Canada.

The carotenoid content was determined spectrophotometrically by the method outlined by Ferrari and Bailey (1929 and 1929a) which is based on the assumption that the principal pigment of wheat flour is carotene. Certain modifications suggested by Dr. Ferrari [6] in a personal communication were employed. The modified method is as follows:

> 20 gms. of flour is weighed into a 250 cc. glass stoppered bottle and 100 cc. of solvent added from a pipette. The bottle is shaken frequently for one hour and allowed to stand in the dark overnight. In the morning it is reshaken, allowed to settle, and the supernatant liquid decanted into 100 cc. lipless centrifuge tubes, closed with rubber caps, and centrifuged at approximately 2200 r.p.m. for 30 minutes. A centrifuge equipped with a brush release was employed. The clear extract is siphoned off with a capillary bore siphon having the shorter end bent upwards and the transmittancy determined in a 10 cm. cell at wave length 4358 Ångström units. The solvent consists of a mixture of 93 parts by volume of a petroleum distillate known commercially as varnish makers' naphtha with 7 parts by volume of absolute ethyl alcohol.

The distillation range of the naphtha used is given in Table I.

Gasoline colour values were determined on the extracts after spectrophotometric determination of carotene by comparison with a series of potassium chromate standards prepared by dilution with distilled water from an 0.5% solution of the pure salt. The comparisons were made

[6] Since the preparation of this manuscript a paper embodying these modifications has been published. (C. G. Ferrari, Cereal Chem. **10**: 277–286. 1933.)

TABLE I

DISTILLATION RANGE OF NAPHTHA

Initial boiling point 316° F.

Temperature range		Per cent distilled over
° F.	° F.	%
316 to	324	10
324 "	326	10
326 "	328	10
328 "	330	10
330 "	333	10
333 "	336	10
336 "	340	10
340 "	346	10
346 "	355	10
355 "	398 (end-point)	9
Residue		0.5
Loss		0.5

at a constant depth of 80 mm. in a special form of Nessler tube made by cementing 25 mm. polariscope cover glasses to the base of glass tubes of the same bore with the aid of hard DeKhotinsky cement. Observations were made vertically through the column of liquid, the tubes standing on a plate of clear glass, illumination being obtained from beneath by means of a metal reflector which was coated with flat white paint and placed at a forty-five degree angle. The source of the illumination for daylight readings was a northeast window and, in the case of the mercury arc, a Hanovia alpine model sun-lamp enclosed in a wooden housing provided with a suitable aperture screened with clear glass.

COMPARISON OF DAYLIGHT AND MERCURY ARC RADIATION

A comparison of daylight and mercury arc radiation as sources of illumination from the standpoint of differentiation between flours and variability between operators was first made. For this purpose extracts of 5 unbleached and 7 bleached flours were prepared, and the gasoline colour value of each extract determined by 5 observers, each using the two light sources consecutively. The data were subjected to an analysis of variance as developed by Fisher (1931). In making the computations, the results for the two light sources and for the bleached and unbleached flours were treated separately. In each instance the variances due to " differences between samples " and " differences between observers " were compared with that of error by the Z test. The statistical data are summarized in Table II.

The variance " differences between samples " measures the variability of the sample means, that is, of the mean gasoline colour values obtained by the five observers on each sample. If this variance is not significantly greater than that of error it can be concluded, in general, that no real differences between the samples exist or that the test has

not been sufficiently accurate to bring out differences in gasoline colour value. The actual comparison of the estimates of variance is made by the application of Fisher's test. If the Z value exceeds the 5% point (20:1 odds) it may be regarded as significant. Standard errors were computed directly from the variance tables. For a single determination the standard error is the square root of the error estimate of variance. From this value the standard errors of the general mean, and of the means for samples and observers is computed by dividing by the square root of the respective number of determinations involved. In comparing the means for samples or observers, differences of less than three times their standard error may be ascribed to chance.

Considering first the data for the unbleached flours, it will be noted

TABLE II

ANALYSES OF VARIANCE, WITH MEANS, FOR GASOLINE COLOUR VALUES AS READ BY DIFFERENT OBSERVERS USING DAYLIGHT AND MERCURY ARC AS ILLUMINANTS

UNBLEACHED FLOURS

Variance due to	Sum of squares	Degrees of freedom	Variance	Z	5% pt.
Daylight					
Differences between samples	0.630	4	0.1575	0.1556	0.5505
Differences between observers	1.644	4	0.4110	0.6352	0.5505
Error	1.846	16	0.1154		
Total	4.120	24			
Mercury arc					
Differences between samples	0.2074	4	0.05185	1.4730	0.5505
Differences between observers	0.0114	4	0.00285	0.0225	0.5505
Error	0.0436	16	0.00272		
Total	0.2624	24			

	Standard error	Mean	S.E. in % of mean
Daylight	0.3397	1.680	20.22
Mercury arc	0.0522	0.848	6.16

Means for observers

Illuminant	Observer A	B	G	J	R	S.E. for observers	General mean	S.E.
Daylight	1.90	1.92	1.84	1.42	1.32	0.152	1.680	0.068
Mercury arc	0.88	0.86	0.83	0.82	0.85	0.023	0.848	0.010

Means for samples

Illuminant	Samples 21	22	23	25	26	S.E. for samples	General mean	S. E.
Daylight	1.76	1.92	1.66	1.44	1.62	0.152	1.680	0.068
Mercury arc	0.98	0.89	0.88	0.75	0.74	0.023	0.848	0.010

BLEACHED FLOURS

Variance due to	Sum of squares	Degrees of freedom	Variance	Z	5% pt.
Daylight					
Differences between samples	0.4954	6	0.0826	1.0013	0.4598
Differenccs between observers	0.1725	4	0.0431	0.6765	0.5106
Error	0.2675	24	0.0112		
Total	0.9354	34			
Mercury arc					
Differences between samples	0.2658	6	0.0443	1.5879	0.4598
Differences between observers	0.0110	4	0.0028	0.1982	0.5106
Error	0.0444	24	0.0018		
Total	0.3212	34			

	Standard error	Mean	S. E. in % of mean
Daylight	0.1056	0.7314	14.4
Mercury arc	0.0430	0.4931	8.7

Means for observers

Illuminant	Observer A	B	G	J	R	S. E. for observers	General mean	S. E.
Daylight	0.786	1.814	0.743	0.700	0.614	0.047	0.731	0.018
Mercury arc	0.500	0.507	0.514	0.471	0.473	0.019	0.493	0.007

Means for samples

Illuminant	Sample number 56	57	58	59	60	61	62	S. E. for samples	General mean	S. E.
Daylight	0.66	0.74	0.68	0.74	0.56	0.98	0.76	0.040	0.731	0.018
Mercury arc	0.40	0.53	0.45	0.54	0.39	0.66	0.49	0.016	0.493	0.007

from Table II that, using daylight as the source of illumination, the mean gasoline colour values of the 5 samples are not significantly different, while the mercury arc results show that real differences exist. For daylight, the mean values of the samples range from 1.44 to 1.92 or through 0.48 units of gasoline colour value and, for the mercury arc, from 0.74 to 0.98 or through 0.24 units. The corresponding standard errors are 0.152 and 0.023, so that the 50% reduction in spread between the samples is accompanied by approximately a seven-fold reduction in error, with the result that the samples are more clearly differentiated by the use of the mercury arc.

In regard to the variance between observers, the reverse is the case, the 5 analysts obtaining essentially similar mean values by the mercury arc and significantly different results with daylight as the light source. For the mercury arc, the means of observers range from 0.82 to 0.88 or a spread of 0.06 units while the daylight readings show a range from 1.32 to 1.90 or 0.58 units. The high variability between observers in

the instance of the daylight readings is due to the brown hue of the flour extracts, which must be mentally disregarded in order to secure any sort of match with the potassium chromate standards.

When flour extracts are observed by radiation from the mercury arc however, the brown hue is largely eliminated and the colour so closely approximates the standards that a much more precise match can be made.

The statistical data for the bleached flours also show that the mercury arc gives a better differentiation between samples, lower variability between observers, and a much lower experimental error. The error variances have been compared by the Z test, and the reduction in random error was found to be highly significant.

Relation Between Carotene and Gasoline Colour Value

The greater precision with which gasoline colour values could be determined by the mercury arc as a light source suggested that these values might be more highly correlated with carotenoid pigmentation as determined by the spectrophotometer. Carotene determinations were accordingly run on a series of 138 experimentally milled flours, 69 of which were bleached with Novadelox. Gasoline colour values, using the two light sources, were run on the same extracts as were employed for the transmittancy measurements. The carotene content of the flours ranged from 0.86 p.p.m. to 2.98 p.p.m. and the correlations between carotene and gasoline colour value were $+0.883$ and $+0.920$ for the daylight and mercury arc readings respectively. The significance of the difference between these correlations was estimated by computing the difference in their corresponding Z values and comparing this with its standard error. The Z values differed by 0.199 ± 0.122 and hence approach significance. While there is not sufficient evidence to conclude that the mercury arc values would in general be more highly correlated with carotenoid pigmentation, the advantages of this light source from the standpoint of ease of matching and lower error variance render it preferable to daylight.

Moreover, the correlation is sufficiently high to indicate that the gasoline colour test may serve as a fairly reliable measure of carotenoid content, and suggests the possibility of expressing the arbitrary gasoline colour value units in terms of carotene concentration.

Spectral Characteristics of Flour Extract and Colorimetric Standards

Before proceeding further, it was deemed advisable to compare the suitability of several suggested standards. Of these, potassium chromate, either alone or with the addition of cobalt nitrate (Kent-Jones, 1927), or a mixture of naphthol yellow and orange G (Sprague, 1928)

were investigated. The main requisites of a colorimetric standard of this type are (1) spectral characteristics similar to those of the material under examination, (2) ease of reproducibility and (3) permanence.

The spectral distribution curves of these standards and of a composite flour extract were determined and are given in Figure 1. The

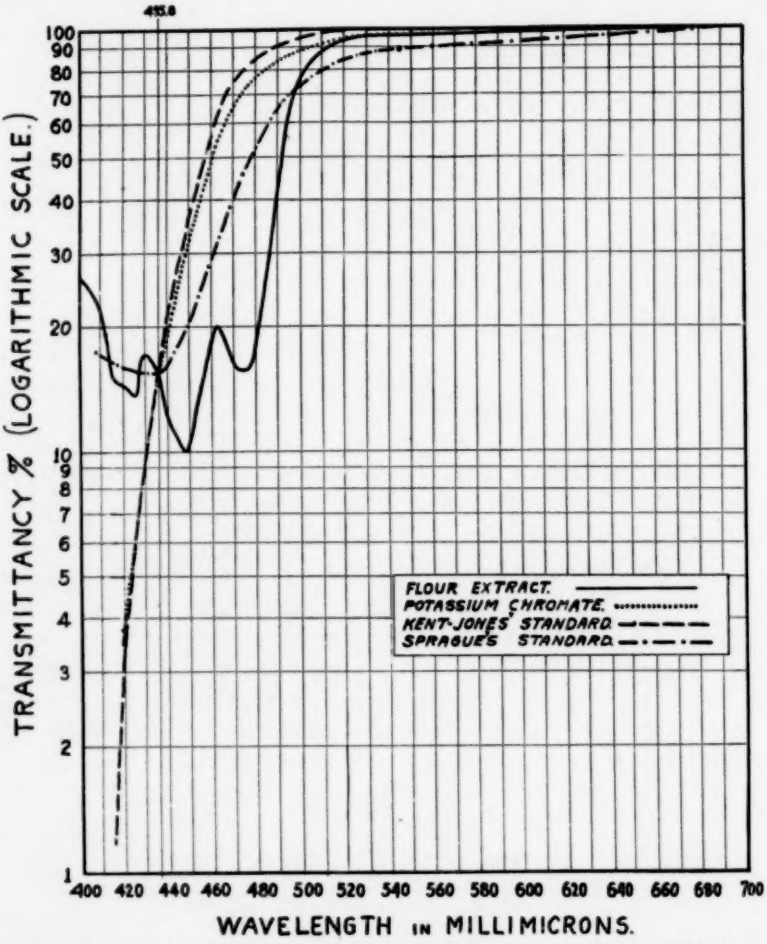

Fig. 1. Comparative spectral properties of flour extract and the following colorimetric standards—potassium chromate, potassium chromate + cobalt nitrate (Kent-Jones' standard) and naphthol yellow + orange G (Sprague's standard).

solutions were prepared with distilled water and diluted to the same transmittancy as the flour extract at 4358 Ångström units in order to obtain a common basis of comparison. This wavelength was selected for the reason that it represents a spectral region in which the various standards and flour extract possess high absorption and is readily available at high intensity in the spectrum of the mercury arc.

The principal differences to be observed are in the region lying between 4400 to 5200 Ångström units. These, together with the variable absorption in the extreme violet, account for the differences in hue between flour extract and the suggested standards when viewed with white light. If daylight is employed, Sprague's standard most closely resembles the colour of flour extract. The addition of cobalt nitrate does not produce a marked alteration in the spectral characteristics of potassium chromate solution.

The curves for potassium chromate and flour extract reveal the reason for the similarity in colour when viewed with mercury arc radiation, which, in the visible spectrum, shows four principal lines, namely, a violet line at 4358 Ångström units, an intense green line at 5461 Ångström units, and two closely adjacent yellow lines at 5770 and 5790 Ångström units respectively. The violet line, 4358 Ångström units, centers a narrow region of high absorption in which the two curves cross. The green and yellow lines intersect the spectral distribution curves at points where they are essentially similar and where the absorption is low, hence the presence of these radiations does not induce appreciable colour differences between the potassium chromate and the flour extract. The colour of the two solutions thus becomes a greenish yellow, the intensity of which is governed almost entirely by the absorption at 4358 Ånström units. It is therefore unnecessary to filter out the green and yellow lines which, while giving a blue colour, would reduce the intensity somewhat. The use of the mercury arc definitely eliminates any transmission of light in the region 4400 Ångström units to 5200 Ångström units which is principally responsible for the colour differences in white light.

From the standpoint of identity of spectral characteristics, Sprague's standard would appear to be preferable for use with white light, whereas with the mercury arc all the standards examined are more nearly of equal merit. On the score of reproducibility and permanence, organic dyestuffs are much less satisfactory than inorganic salts. These points have been studied by Sprague and Troxler (1930) who found distinct variations in the purity of naphthol yellow obtained from different sources, and suggest that a large quantity of the dye be purchased at one time and standardized against pure carotene. The working standards must also be prepared frequently from concentrated stock solutions which themselves must be discarded upon the appearance of a precipitate.

With reference to Kent-Jones' standard, as the addition of cobalt nitrate produces no appreciable alteration in the spectral characteristics of potassium chromate, its use in conjunction with the mercury arc as a light source does not appear to offer any advantage and, in addition, complicates the preparation of the standard. All things considered,

potassium chromate appears to be the most satisfactory standard of those investigated. It contains no water of crystallization, is not hygroscopic, and is readily obtainable in a state of high purity.

Influence of H-ion Concentration on Colour of Potassium Chromate Solutions

In the experiments so far reported the potassium chromate standards were prepared with distilled water. However, Jørgensen (1927) observed qualitatively that the colour of such solutions is markedly influenced by changes in H-ion concentration, the chromate acting as a feeble pH indicator, the effect being most apparent between pH 6 and pH 7. To secure quantitative data on this point a series of 0.9 gasoline colour value standards (0.0045 gms. K_2CrO_4 per 100 cc.) were prepared in M/20 buffer solutions (Clark and Lubs (see Clark, 1928)) ranging from approximately pH 5.0 to pH 9.0, the actual values being determined electrometrically. The transmittancy of each solution was read at a wave-length of 4358 Ångström units employing the corresponding buffer solution in the solvent cell of the spectrophotometer. The results are represented graphically in Figure 2 and show a decrease in transmit-

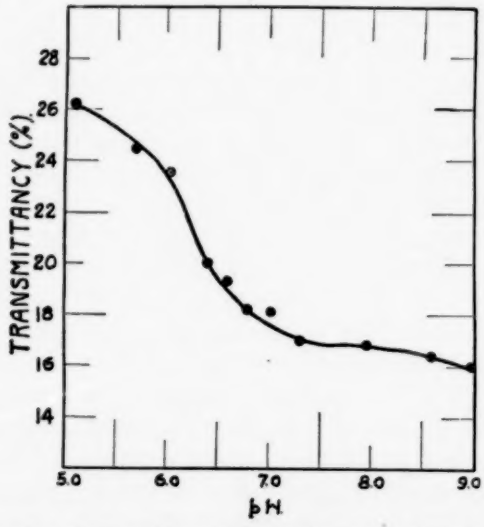

Fig. 2. Showing the effect of pH on the transmittancy of potassium chromate solution (0.9 gasoline colour value) at 4358 Ångström units.

tancy with increasing pH, the change being greatest in the region pH 6 to pH 7, thus confirming the observations of Jørgensen (1927). Within the region pH 7.3 to pH 8.5 the change in transmittancy is less than 1% and is not discernible by the eye.

The H-ion concentrations of a series of potassium chromate standards prepared with distilled water are recorded in Table III. The pH ranges

TABLE III

HYDROGEN-ION CONCENTRATION OF POTASSIUM CHROMATE STANDARDS PREPARED WITH DISTILLED WATER OF pH 6.84[1]

Gasoline colour value	pH
0.20	7.42
.40	7.55
.60	7.52
.70	7.57
.80	7.44
.90	7.47
1.00	7.52
1.10	7.60
1.20	7.57
1.40	7.61
1.60	7.64
1.80	7.70
2.00	7.73
2.20	7.78
2.40	7.80
2.60	7.75
2.80	7.84
3.00	7.88

[1] pH was determined electrometrically with the quinhydrone gold electrode.

from 7.42 to 7.88 for gasoline colour values of 0.2 to 3.0. These pH values fall within the flat portion of the pH-transmittancy curve and therefore such variations have no appreciable effect on results obtained with these standards.

It might be regarded advisable, however, as an extra precaution, to employ a buffer solution in order to ensure that the H-ion concentration always lies within the flat portion of the curve. A series of standards were accordingly prepared at a pH of 7.64 using M/20 KH_2PO_4—NaOH with the object of checking their stability by means of transmittancy measurements over an extended period. Within a period of one month all these solutions had developed mould to a varying extent rendering this impossible. In view of the fact that standards made with distilled water have been found by Bovis (1928) to remain substantially constant for several years, and also of the insignificant influence of the variations in pH occurring in such standards, it seemed unnecessary to proceed any further in this direction. Standards made with distilled water in this laboratory have been kept without special precautions for three years without growth development. Results obtained with these solutions are in satisfactory agreement with freshly prepared standards.

The potassium chromate standards employed in the original series

of comparisons of carotene concentration and gasoline colour value may be thus regarded as satisfactory.

Relation Between Carotene Concentration and Gasoline Colour Value

In order accurately to establish the relationships between carotene and gasoline colour value the original series of comparisons was extended to a larger number of samples with a wider range in carotene content and using only the mercury arc as a light source. In all, 358 samples were employed. The results are summarized by the statistical constants recorded in Table IV and in the form of a regression graph

TABLE IV

Statistical Constants for Carotene and Gasoline Colour Value Data Obtained on the Same Flour Extracts
($N = 358$)

	Carotene (c)	Gasoline colour value (g)
Mean	2.191	0.918
Standard deviation	0.770	0.336
Correlation (carotene × gasoline colour) r_{cg}	+.985	5% pt. = .103
Regression coefficient of carotene on gasoline colour	=2.2571	
Regression equation for computing carotene from gasoline colour value	$c = 0.1205 + 2.2571\ g$	

given in Figure 3.

The close relationship of gasoline colour value and carotene is shown by the high and significant correlation of $r = +0.985$ (5% pt. 0.103). As $(1 - r^2)$ may be regarded as an estimate of the fraction of the total variance of carotene independent of gasoline colour value, 97% of the variance is attributable to the correlation. Hence, comparison with potassium chromate standards using the mercury arc as an illuminant may be employed as a reliable estimate of carotene concentration, by having recourse to the regression function, which represents the most probable value of carotene for any given gasoline colour value.

In computing the regression equation, the carotene values were grouped in classes covering a range of 0.15 p.p.m. carotene. The regression line is shown in Figure 3 with the frequencies for each colour value and the actual means and frequencies for each carotene class. With the exception of the higher values, for which the frequencies are low, the average carotene values fall very close to the regression line. The linearity of regression has been tested by the analysis of variance procedure (Fisher, 1931) and the deviations from the regression line were not significant.

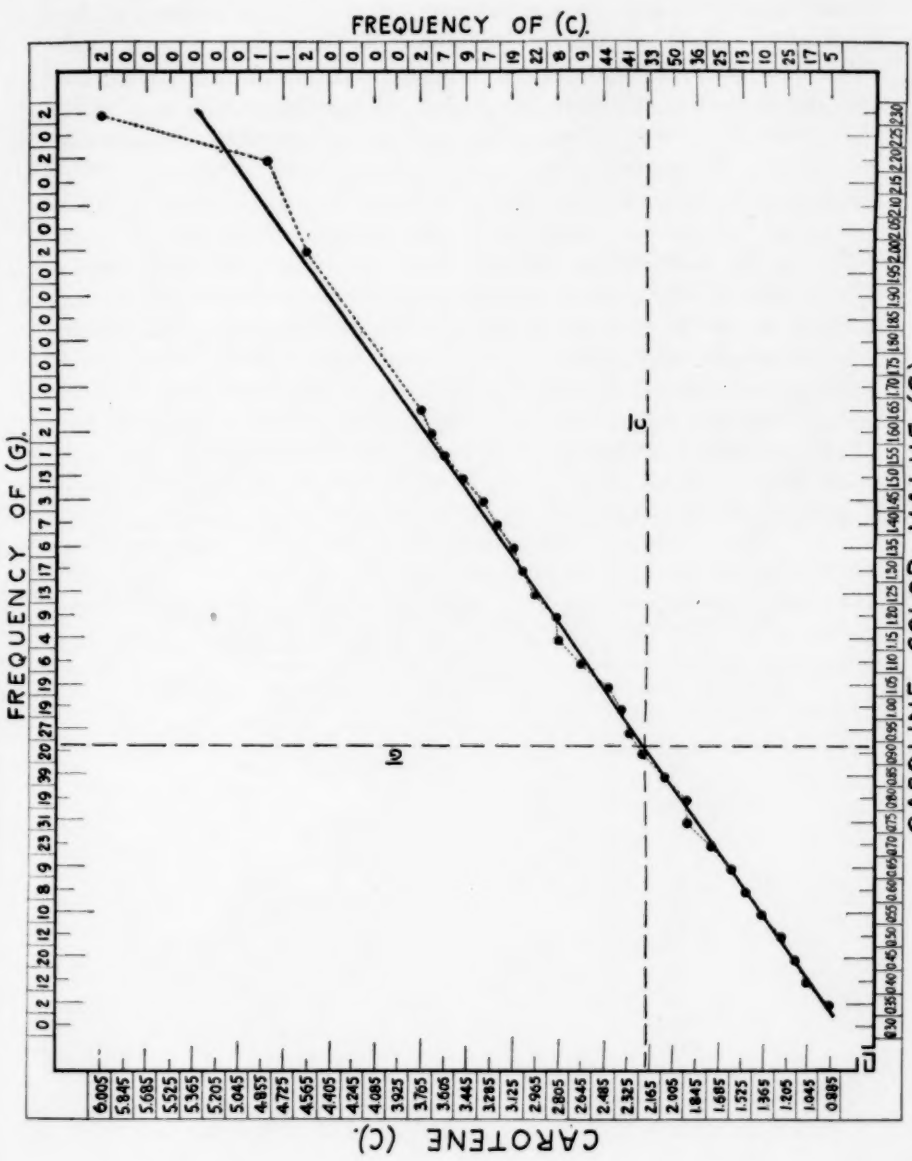

Fig. 3. Regression of carotene on gasoline colour value.

The regression equation given in Table IV ($C = 0.1205 + 2.2571\ G$ where C = carotene concentration in p.p.m. of flour and G = gasoline colour value) may therefore be employed to compute the carotene equivalent of any given gasoline colour value. These equivalents were calculated for gasoline colour values ranging from 0.35 to 1.65. Carotene was determined spectrophotometrically and colorimetrically employing the calculated carotene values of the conventional gasoline colour value standards. The results by the two methods showed excellent agreement which was maintained over a series of such comparisons until a fresh supply of solvent was used which gave consistently higher carotene values by the colorimetric method. This was finally traced to colour in the solvent which obviously affected only the colorimetric results since, in the spectrophotometric method any colour effect of the solvent is automatically eliminated by the technique employed. In developing a colorimetric method for determining carotene, therefore, it is essential that this variable of solvent colour be eliminated. This may be readily accomplished by redistillation. The use of redistilled solvent, however, made it necessary to apply a correction factor to the original carotene equivalents. The magnitude of this correction was determined with

TABLE V

CAROTENE EQUIVALENTS (COMPUTED FROM THE MODIFIED REGRESSION EQUATION) FOR GASOLINE COLOUR VALUES USING THE MERCURY ARC AS A LIGHT SOURCE

Gasoline colour value	Carotene equivalent
	p.p.m.
0.35	0.77
.40	0.89
.45	1.00
.50	1.11
.55	1.22
.60	1.34
.65	1.45
.70	1.56
.75	1.68
.80	1.79
.85	1.90
.90	2.01
.95	2.13
1.00	2.24
1.05	2.35
1.10	2.47
1.15	2.58
1.20	2.69
1.25	2.80
1.30	2.92
1.35	3.03
1.40	3.14
1.45	3.26
1.50	3.37
1.55	3.48
1.60	3.59
1.65	3.71

an extended series of comparisons, the mean carotene content by the colorimetric method being 0.1375 p.p.m. higher than by the spectrophotometric method. The regression equation given in Table IV therefore becomes $C = -0.017 + 2.2571\,G$ where C = carotene concentration in p.p.m. of flour and G = gasoline colour value.

This modified equation was used to compute the carotene equivalents for the gasoline colour values shown in Table V.

Determination of Carotene by the Modified Gasoline Colour Value Method

If the comparisons are to be made by means of a variable depth colorimeter, any suitable concentration of the gasoline colour value standard may be employed. If, however, the comparisons are made by the standard series method using Nessler tubes, it would be preferable to prepare the standard solutions with values corresponding to even units of carotene. This is particularly desirable as matching with such a series frequently involves interpolation between standards. It will be noted from Table V that the carotene equivalents for the conventional gasoline colour value units, while progressing by uniform increments, give irregular values, thus complicating the interpolations. The concentrations of potassium chromate required to produce standards of uniform carotene values and differing by increments of 0.2 p.p.m. were accordingly calculated and are presented in Table VI. The carotene

TABLE VI

Volume in cc. of 0.5% Potassium Chromate Solution per 1000 cc. Required to Produce Standards Equivalent to 0.6 to 4.2 Parts per Million Carotene (in Increments of 0.2 p.p.m.) in Flour [1]

Carotene value	0.5% potassium chromate per 1000 cc.
p.p.m.	*cc.*
0.60	2.73
0.80	3.62
1.00	4.51
1.20	5.39
1.40	6.28
1.60	7.16
1.80	8.05
2.00	8.94
2.20	9.82
2.40	10.71
2.60	11.59
2.80	12.48
3.00	13.37
3.20	14.25
3.40	15.14
3.60	16.02
3.80	16.91
4.00	17.80
4.20	18.68

[1] Based on a sample to solvent ratio of 20 gms. flour to 100 cc. solvent.

values given in the table are expressed in parts per million of flour based on a solution containing the extractive from 20 gms. flour in 100 cc. solvent. In case it is desired to express the carotene content of a solution without reference to the material from which it was extracted, the above values divided by 5 read milligrams of carotene per liter of solvent.

The comparisons may be made, as indicated in the methods of analysis of the A. A. C. C. (1928), by using a colorimeter or in Nessler tubes employing a series of standards. It has been the authors' experience that colorimeters of the variable depth type are not at all satisfactory for this particular purpose. The use of a series of standards in suitable tubes eliminates much of the uncertainty in placing the unknown at its proper value. When a series of fixed standards is employed, differing by small increments, it is possible to place the unknown in juxtaposition with standards of lower and higher concentration, thus providing limits within which the sample may be readily placed. With a plunger type of colorimeter, the eye has only one reference point against which the sample possesses a varying value upon alteration of depth, which renders accurate matching difficult.

TABLE VII

COMPARATIVE VALUES FOR CAROTENE CONCENTRATION IN FLOUR AS DETERMINED BY THE SPECTROPHOTOMETER AND THE POTASSIUM CHROMATE METHOD USING A STANDARD SERIES AND A DUBOSCQ COLORIMETER

	Carotene in flour					
		Potassium chromate method			Deviations from spectrophotometer values	
Sample number	Spectrophotometer (a)	Standard series (b)	Duboscq colorimeter (c)	Setting of unknown	Standard series (b-a)	Duboscq colorimeter (c-a)
	p.p.m.	*p.p.m.*	*p.p.m.*	*mm.*	*p.p.m.*	*p.p.m.*
1	1.07	1.05	1.15	50	−.02	+.08
2	1.29	1.20	1.37	50	−.09	+.08
3	1.52	1.55	1.58	50	+.03	+.06
4	1.66	1.65	1.63	40	−.01	−.03
5	1.95	1.90	1.92	40	−.05	−.03
6	2.13	2.10	1.91	30	−.03	−.22
7	2.32	2.30	2.25	30	−.02	−.07
8	2.56	2.55	2.28	30	−.01	−.28
9	2.63	2.75	2.75	30	+.12	+.12
10	2.90	2.90	2.96	30	.00	+.06
11	3.08	3.10	3.32	30	+.02	+.24
12	3.27	3.35	3.52	30	+.08	+.25
13	3.42	3.45	3.51	30	+.03	+.09
14	3.57	3.65	3.68	30	+.08	+.11
15	3.82	3.85	4.19	30	+.03	+.37
16	4.02	3.95	4.25	30	−.07	+.23
Mean	2.576	2.581	2.642		+.0056	+.0662

In order to obtain information as to the relative accuracy of the two methods of comparison and, at the same time, to check the reliability of the modified chromate standards for the determination of carotenoid pigmentation in flour extract, the following experiment was conducted. A flour extract of high carotenoid content was diluted to produce a series of 16 solutions ranging in terms of carotene content of flour, from approximately 1 to 4 p.p.m. The carotene content of each extract was determined spectrophotometrically and colorimetrically, using the two methods of comparison outlined above. Mercury arc illumination was employed in both cases. For the variable depth method a 50 mm. solid plunger type Duboscq colorimeter was used, the potassium chromate standard having a carotene value of 4.00 p.p.m. To simplify calculation, the unknown, rather than the standard, was set at a series of fixed depths as suggested by Yoe (1928). Ten settings of the standard cup were made on each solution. The results are given in Table VII and paired analyses of variance for the results by each colorimetric method in comparison with the spectrophotometer are summarized in Table VIII.

TABLE VIII

ANALYSES OF VARIANCE FOR DATA GIVEN IN TABLE VII

Source of variance	Sum of squares	Degrees of freedom	Variance	Z	5% point
Comparison of Spectrophotometer (a) and Standard Series Values (b)					
Between methods {(a) and (b)}	0.0002	1	0.0002	—	0.7568
Between pairs	26.0147	15	1.7343	3.4972	0.4374
Within pairs	0.0238	15	0.0016		
Total	26.0387	31			
Means					
Spectrophotometer 2.576		Standard series 2.581		Standard error of means 0.010	
Intra-class correlation for "between pairs"			$r = 0.998$	5% pt. = 0.4374	
Comparison of spectrophotometer (a) and Duboscq colorimeter (c) values					
Between methods {(a) and (c)}	0.0351	1	0.0351	0.4506	0.7568
Between pairs	27.9894	15	1.8660	2.4371	0.4374
Within pairs	0.2139	15	0.0143		
Total	28.2384	31			
Means					
Spectrophotometer 2.576		Duboscq 2.642		Standard error of means 0.0282	
Intra-class correlation for "between pairs"			$r = 0.985$	5% pt. = 0.4374	

The variance "between methods" is not significant in either instance and hence the mean value by both colorimetric methods may be regarded

as in essential agreement with that given by the spectrophotometer. In other words, the mean deviations of + 0.0056 and + 0.0662 from the spectrophotometer values for the standard series and Duboscq results respectively are not significant.

However, it will be observed that the error variance " within pairs " is much higher for the comparison involving the Duboscq results. The Z value for the difference in error variance is 1.2058. As the 5% point is 0.4374 the difference is highly significant. When the greater error of the Duboscq method is considered in conjunction with the fact that the readings may be made by comparison with a standard series in a fraction of the time, and without the necessity of calculation, the superiority of the latter method becomes apparent.

Hitherto, one of the chief objections to the standard series method has been the variable nature of ordinary Nessler tubes. The bottoms of these tubes being polished on one side only are irregular in figure thus introducing distortion when a column of liquid is viewed vertically. Furthermore, they are calibrated to a definite volume and while obtainable in matched sets, the customary tolerance in height of graduations (6 mm.) is too great for this type of work. These difficulties have been met in this study by the use of tubes with cemented on plane-parallel bottoms and graduation marks at a fixed depth of 80 mm. In routine service the cementing material is apt to loosen and tubes have recently been especially made for this work with fused on plane-parallel bottoms.[7]

Discussion

The experimental data presented deal only with a comparison of the carotenoid content of flour extracts as determined spectrophotometrically and colorimetrically. Factors involved in the preparation of the extract are being made the subject of a separate study and are not pertinent to the relationships revealed in this investigation. On the assumption that the pigment of flour extract is substantially carotene, the carotene equivalents of potassium chromate solutions may be used for the determination of this pigment in extracts of other biological materials. Preliminary studies in this laboratory indicate that xanthophyll together with a third unidentified pigment is also presnt in flour extract, but quantitative data are not yet available on this point. However, as pointed out by Ferrari and Bailey (1929), the spectral properties of xanthophyll and carotene are so similar that the presence of a considerable quantity of the former pigment would not introduce an appreciable error into the spectrophotometric results which have been used as a basis for determining the carotene equivalents of potassium chromate solutions.

[7] Eck and Krebs, 131 West 24th Street, New York, N. Y.

The fundamental basis of the simplified method is the use of mercury arc radiation as an illuminant for the colorimetric comparison of flour extracts obtained with colourless redistilled solvent. The carotene equivalents given were obtained in this way and are not applicable to values with other light sources or solvent that is not entirely colourless. The use of light filters in conjunction with a white light source is not feasible on account of the great reduction in intensity and the relatively broad spectral regions transmitted. The suitability of the General Electric Sun Lamp for this purpose was investigated. It was found to effect only a slight improvement over white light in the elimination of the brown hue of flour extracts, due to the fact that the mercury arc spectrum is superimposed on a continuous background produced by the incandescent filament of the lamp. It is not at all essential that a "quartz" mercury arc be used and there are available a number of inexpensive arcs of the capillary type constructed of hard glass which are equally satisfactory and are free from excess ultra-violet radiation. The only additional equipment required is a simple Nessler comparator as previously described and a suitable number of plane-parallel bottom comparison tubes.

The standards suggested (0.60 to 4.0 p.p.m.) amply cover the normal range in carotene content of bleached and unbleached flours. If lower values are to be determined, the appropriate standards may be calculated from the regression equation and the comparisons made at a greater depth than 80 mm. For values exceeding 4.0 p.p.m. it is desirable to employ the appropriate standards in a depth of 40 mm.

The method has been satisfactorily employed in determining the carotene content of extracts prepared from finely ground wheat. The selection of a suitable solvent which will yield results on wheat comparable to those obtained on the flour milled therefrom is also under investigation.

Summary

It has been observed that the difference in hue of gasoline extracts of flour and aqueous solutions of potassium chromate employed in the Winton gasoline colour value determination is almost entirely eliminated when mercury arc radiation is substituted for white light as an illuminant.

While the change in illuminant reduced the gasoline colour values by approximately fifty percent, with a consequent reduction in spread between samples, this was more than offset by the greater precision attainable in matching, the use of mercury arc radiation giving a greater differentiation between flours, lower variability between observers and a significantly lower random error.

A determination of the spectral properties of flour extract and potassium chromate solution revealed wide differences in the region lying between 4400–5200 Ångström units where no radiation is emitted by the mercury arc. For both solutions, the violet line at 4358 Ångström units centered a narrow region of high absorption, while the green and yellow lines intersected the spectral distribution curves at points of low absorption. These observations account for the similarity in colour of flour extract and potassium chromate solution when viewed with mercury arc radiation.

The spectral properties of the colorimetric standards suggested by Kent-Jones and by Sprague were not found to be radically different from potassium chromate, which is regarded as the most satisfactory colorimetric standard investigated when permanency and reproducibility are taken into consideration.

The transmittancy of potassium chromate solution decreases with increasing pH, the change being greatest in the region pH 6 to pH 7. Within the region pH 7.3 to pH 8.5 the change in transmittancy is less than one percent and is not discernible to the eye.

The pH of a series of potassium chromate standards made up in distilled water ranged from 7.42 to 7.88 for gasoline colour values of 0.2 to 3.0 respectively. These pH values fall in the flat portion of the pH-transmittancy curve and hence the use of buffers is unnecessary. Standards prepared in a buffer solution of pH 7.64 were found to be unstable.

A comparison of the gasoline colour values obtained by mercury arc illumination with spectrophotometric determinations of carotene on the same flour extracts, in the instance of 358 flours of varying pigment concentration, gave a positive correlation of 0.985 and a straight line regression. The arbitrary gasoline colour value units may thus be expressed in terms of carotene by employing the regression equation.

The carotene equivalents for the Winton gasoline colour standards fall on irregular values and directions are given for the preparation of potassium chromate standards corresponding to even units of carotene. These values are dependent upon the use of mercury arc illumination and colourless redistilled solvent for the preparation of the flour extracts.

The reliability of the modified chromate standards for the determination of carotene, employing both the standard series method using Nessler tubes and a Duboscq colorimeter, has been checked against the spectrophotometric method. An analysis of the data for 16 samples showed that both colorimetric methods gave results in agreement with the spectrophotometric values. The experimental error, however, was significantly higher for the Duboscq data. The standard series method

of comparison, employing special tubes with plane-parallel bottoms calibrated at fixed depths of 40 mm. and 80 mm., is recommended.

While the colorimetric method suggested for the determination of carotene is not susceptible of as great accuracy as the spectrophotometric method, the equipment is much less expensive, the determinations are rapidly and easily made and the method is therefore well suited for routine work.

Acknowledgments

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DIASTATIC ACTIVITY IN DOUGHS AND SUSPENSIONS

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(Read at the Convention, June, 1933)

Although Kirchoff (1815) first observed the enzymic degradation of starch, and the dual nature of the diastatic complex was early recognized by Maerker (1877), the fact that diastatic activity or "gassing power" is an important factor in bread production was not well understood until the pioneer work of Wood (1907), and his contemporaries (Humphries and Biffin (1907), Baker and Hulton (1908), Ford and Guthrie (1908), Neumann and Salecker (1908), Liebig (1909), Alway and Hartzell (1910)). The fact that it is *autolytic* diastatic activity, not saccharifying activity (Lintner, 1886), or dextrinizing activity (Roberts (1881), Wohlgemuth (1908)), that is of significance in baking chemistry was shown by Rumsey (1922) who first developed an index of this activity, even though the autolytic method had previously been studied by Swanson and Calvin (1913). The two factors in autolytic activity, viz., enzyme content and starch susceptibility, were definitely separated by the work of Mangels (1926), Alsberg (1927), and Malloch (1929), although the results were foreshadowed by Stone (1896), Humphries and Simpson (1909), Whymper (1909), Reichert (1917, 1917a), Dox and Roark (1917), Shollenberger and Coleman (1926), and others. Wood, Humphries, and contemporaries regarded diastatic activity as one of the factors of flour strength, and made many attempts to correlate diastatic activity and baking quality. When Rumsey (1922) provided an analytical index of diastatic activity it was found that such correlations were of a very low order and irregular (Collatz and Racke (1925), Sherwood and Bailey (1926), Blish, Sandstedt, and Platenius (1929), Pascoe, Gortner, and Sherwood (1930)). It is now becoming generally recognized (Kent-Jones (1924), Fisher and Halton (1929), Jørgensen (1931), Swanson and Kroeker (1932)) that diastatic activity is an environmental factor, one under the control of

the baker and miller, unrelated to inherent flour properties and to flour strength.[1]

Factors Involved

The results of these investigators have shown that saccharogenic activity[2] is influenced by many factors, the most important of which are diastatic content, d; starch susceptibility, s; H-ion concentration, pH; temperature, T; and time, t. Hence, if "i" represents the initial sugar concentration, the potential sugar level, S_p,[3] for any flour water mixture may be expressed in functional notation as:

$$S_p = F(i, d, s, pH, t, T, \ldots).$$

The problem of determining the form of this function and calculating the potential sugar level at any time, being one of great importance in practical baking as well as of theoretical interest, involves the analysis of flour water mixtures of many compositions and under various conditions.

Methods of Analysis

Both chemical and biological (fermentation) methods have been applied to this problem:

1. *Chemical Methods*

The analysis of yeast-free doughs or suspensions by chemical methods offers no particular difficulty. A determination of sugars both before and after inversion is sufficient to give the sugar level at any time. The A. O. A. C. methods have been found to check well with the fermentation methods described below.

In a fermenting dough, however, not only sucrose and maltose are present, but also varying concentrations of levulose and dextrose. At present sufficiently reliable methods do not appear to be available for a complete analysis of such a complex mixture. Since, however, at the present stage of baking technique it is *fermentable* sugars which are of singular importance, biological methods may be expected to give results satisfactory for our purpose.

2. *Fermentation Methods*

(*a*) *Analysis of Stable Sugar Solutions:* The use of yeast for the direct determination of sugar by measurement of the gas evolved in fer-

[1] A more comprehensive review of the development of the concepts regarding the diastatic enzymes of flour has recently been given by C. N. Frey (1933).

[2] In the remainder of this paper it will be understood that the use of the term saccharogenic activity, unqualified, is to mean autolytic activity only, that is, the activity of the flour or dough enzymes on the starch of the flour or dough itself.

[3] The potential sugar level is here defined as the theoretical sugar concentration expressed as per cent of the flour, for the system under consideration if the fermentation rate were zero, all other conditions being equal. It is the sum of the previously fermented sugars and the actual sugar concentration at any time. The term, unqualified, is taken to mean fermentable sugars.

mentation has been described by Blish, Sandstedt, and Astleford (1932), and Schultz and Kirby (1933). As indicated by Blish *et al.* (1932) it has been found that the pressure generated at constant volume (Fig. 1) is directly proportional, within limits, to the amount of sugar fermented (Fig. 2). Such has also been found to be the case for the

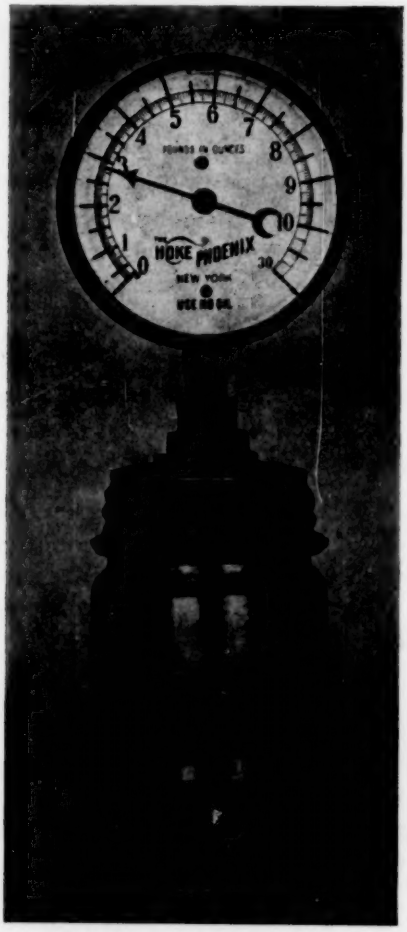

Fig. 1. A convenient device for the analysis of sugar solutions by the biological method.

increase in volume at constant pressure.[4] Most of the data reported herein have been obtained by the following modification of the Schultz and Kirby (1933) method:

[4] A device similar to that described by Markley and Bailey (1932) for automatically measuring and recording at constant pressure the gas generated, has been of invaluable assistance in this study. The device operates upon the principle of compensation for the water displaced by the use of springs, and is essentially a recording gas burette (Vaughen, 1932).

100 cc. of dough filtrate, sugar solution, or bread extract in a pint mason jar is placed in a shaker in a thermostat at 30° C. After temperature equilibrium is attained the jar is removed and the contents brought to a pH in the neighborhood of 4.5 if necessary; 10 cc. of buffer (Schultz and Kirby, 1933), and ½ gm. of peptone are then added. This is followed by 10 gms. of yeast, the solution being vigorously stirred by the use of a high speed drink mixer. Within 15 to 20 seconds the contents are thoroughly mixed. The jar is capped, replaced in the shaker and connected to the recording mechanism. The recording instrument may be calibrated to read directly in grams of sugar (as sucrose).

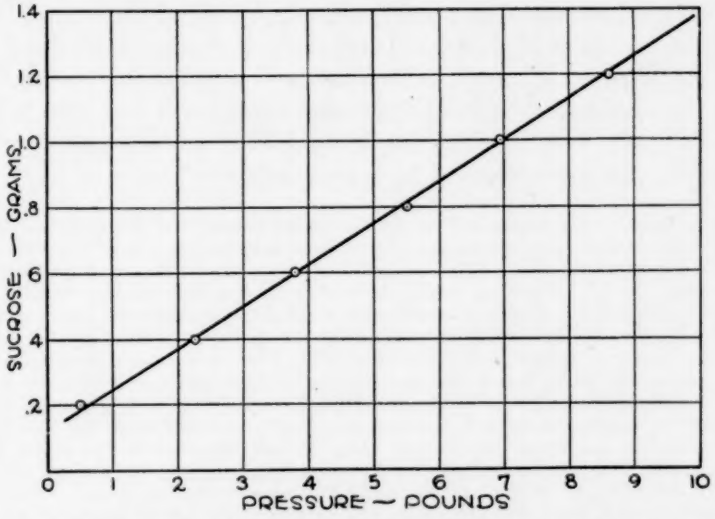

Fig. 2. Calibration curve. Sugar as measured by increase in pressure at constant volume. Pint mason jar of 490 ± 3 cc. capacity, total volume of suspension 90 cc.; yeast, 20 gms.; buffer, 25 cc.; peptone, 0.5 gm.; inert starch, 25 gms. Jar shaken continuously 2 minutes before reading. Pressure registered on a Hoke-Pheonix "Ouncer" gauge.

(*b*) *Analysis of Diastatically Active Solutions:* The method described by Schultz and Landis (1932), in which the sugars are fermented from a diastatically active solution as rapidly as they are formed, has also been applied to this problem. The following modification has been found convenient for determining the sugar level in flour suspensions:

50 gms. of flour, 125 cc. of water (250% absorption), including buffer if desired, ½ gm. peptone, 20 gms. of yeast (40%), and ½ gm. salt (1%) in a pint mason jar are placed in the shaker in the thermostat and allowed to come to temperature equilibrium. The jar is then placed under the high speed mixer and 50 gms. of the flour to be studied is sifted in. It is convenient to add the flour by means of a cornucopia of paper during the beating of the solution. The suspension becomes homogeneous in thirty seconds. It is then replaced in the shaker and connected to the recording instrument. In the case of particularly strong flours it is advisable to interpose a Kjeldahl trap between the jar and the instrument as the batter is likely to produce excessive foam. This method possesses the advantage of indicating the diastatic level at or before one hour (*cf.* Geddes and Winkler (1930), and Schultz and Landis (1932)), while minimizing errors due to the metabolic activity of an excessive amount of yeast (see Blish *et al.* (1932)).

3. *Dough Disintegration*

The solution to the problem of completely inhibiting diastasis in suspensions has been finally completed by Blish *et al.* (1932). The rapid disintegration and clarification of a dough, however, has offered some difficulty (Rumsey (1922), Collatz and Racke (1925), Geddes and Winkler (1930), Blish and Hughes (1932)). An appreciable time interval must usually elapse between the beginning of disintegration and the final inhibition of diastasis, particularly if excessive dilution of the final extract is to be avoided. By means of a power-driven egg beater with the cautious addition of clarifying solutions, it has been possible to reduce the time required for disintegration to one minute or less. The following procedure has been used with some success:

The beaters of a power-driven egg beater are placed in a 100-gram fermenting dough contained in a pint mason jar thirty seconds before the time at which the diastasis is to be inhibited. The jar and beater are placed in a bath of circulating ice water. 50 cc. of normal sulphuric acid is then added very gradually to the dough by means of a dropping funnel with a suitably bent delivery tube. Approximately fifteen seconds should be allowed for the delivery of this quantity of acid. The egg beater is started at medium speed, after 5 to 10 cc. of the solution has been delivered. The dough has a strong tendency to climb the spindles of the beaters, and is continually cut down by the judicious use of a spatula. When all the acid is added the beating is stopped, the beaters partially removed from the solution and any small bits of dough which may have become attached to the inside of the beaters, and thus not subject to disintegration, are removed with a spatula. The beaters are replaced in the solution and the disintegration continued. The dough is meanwhile cut away from the sides and bottom of the jar by means of a spatula and the beaters. Between one-half and one minute is usually required for the major portion of the disintegration. After the suspension assumes a creamy consistency, 50 cc. of solution containing an appropriate amount (25 cc. of 12% is usually sufficient for 100 grams of most flours) of sodium tungstate is added through the dropping funnel. This is followed by 50 cc. of water. 25 cc. of a standard reference solution[5] is then added. All reagents are previously cooled to 1° C. When the suspension is homogeneous, the beaters are removed, and the disintegrate immediately centrifuged. The concentration of reference material may be determined on an aliquot of the supernatant liquor. At this concentration the supernatant liquor is seldom clear, but is suitable for analysis by method 2*a*. 10 cc. of normal sodium hydroxide per 100 cc. of dough centrifugate, with the addition of 10 cc. of buffer solution, has been found to produce a suitable medium for fermentation.

Sugar Level in Buffered Suspensions and Yeast-free Doughs as a Function of Time

Some results obtained by the application of these methods are indicated in Figures 3, 4, 5, and 6. It is apparent that under these condi-

[5] As noted by Newton and Cook (1930) and others, the amount of water held by the dough colloids may amount to as much as 40% of the flour. Doubt has been recently cast upon this value by Greenberg and Greenberg (1933), who found by actual analysis of ultra filtrates, not by cryoscopy, that practically all of the water in a hydrophillic sol is available for solvent action. Hence an inert reference substance has been added to determine the amount of flour represented by an aliquot of the dough centrifugate. Suitable reference substances have been found to be magnesium chloride and sulphuric acid. Sodium oxalate gave erroneous results. It is assumed that the sugars will be distributed throughout the solution in the same way as the reference materials.

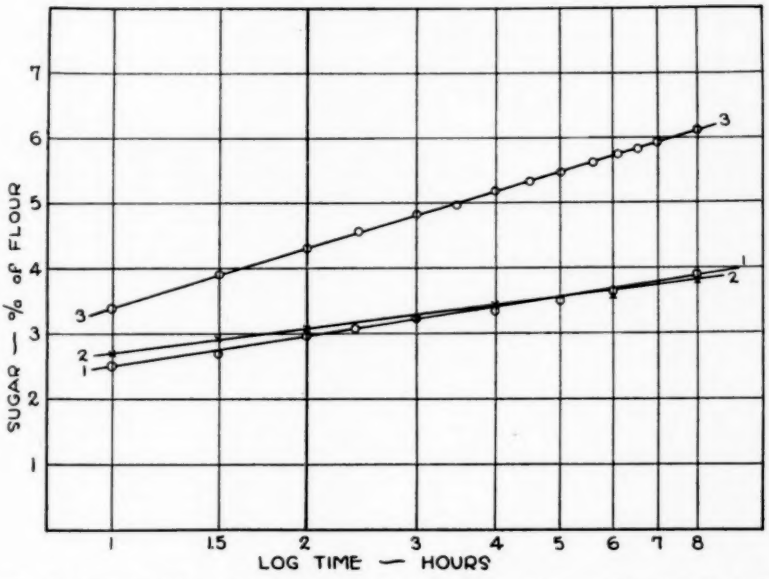

Fig. 3. Sugar level in yeast-free doughs and in buffered suspensions. Northwestern hard wheat patent flour. (1) Yeast-free dough, 60% absorption. (2) Suspension at 250% absorption. (3) Sugar level by method 2*b*, pH 4.7–4.9.

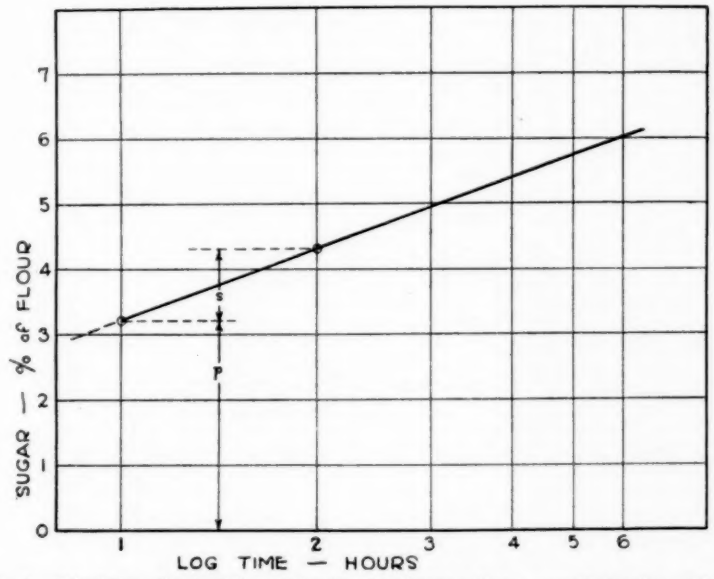

Fig. 4. Calculation of potential sugar levels. p, primary value; s, secondary value. The equation of the line is $Sp = (s/\log 2) \log t + p$. A thread stretched across the chart on the points as indicated will show the potential sugar level at any time.

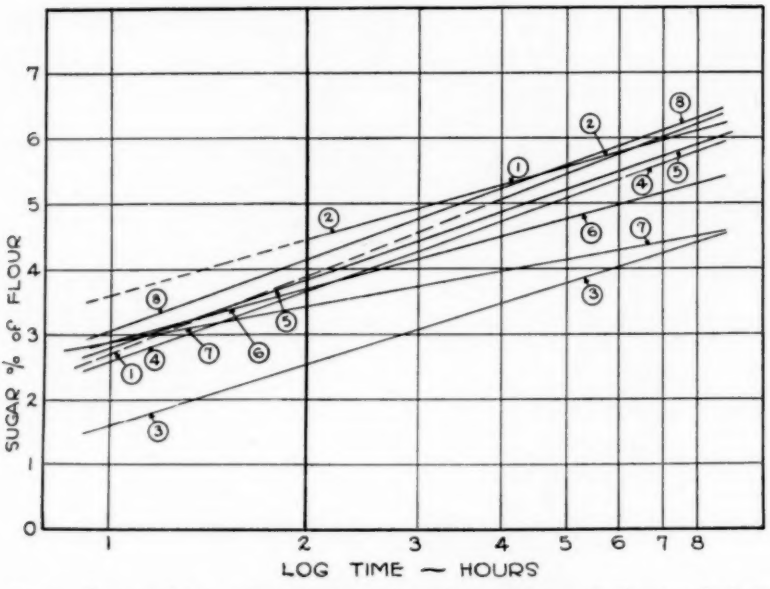

Fig. 5. The potential sugar level function under various conditions. Northwestern hard patent flour. (1) (circles) 2% yeast sponge dough. (2) 10% yeast sponge dough. (3) method *2b*, average pH 6.5. (4) method *2b*, average pH 5.4. (5) method *2b*, average pH 4.8. (6) method *2b*, average pH 4.4. (7) method *2b*, average pH 4.2. (8) method *2b* unbuffered, average pH 4.7.

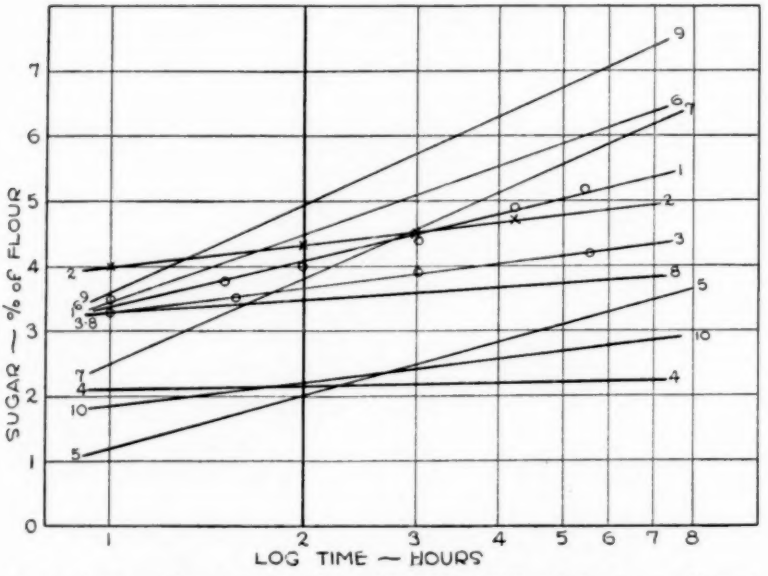

Fig. 6. Some typical saccharogenic values. (1) Collatz (1922), reducing sugars, arbitrary index. (2) Berliner and Ruter (1930), reducing sugars, arbitrary index. (3) Gore (1932), polariscopic determination, arbitrary index. (4) Experimentally milled Indiana straight flour. (5) Experimentally milled southwestern clear flour. (6) Northwestern hard wheat patent flour. (7) Manitoba hard red spring wheat flour. (8) Oklahoma, 1931 hard patent flour. (9), (10) Collaborative.

tions the sugar concentration after one hour is a logarithmic function of the time and may conveniently be expressed by the equation:

$$S_d = \frac{s}{\log 2} \log t + p,$$

where p is the per cent sugar at one hour (the intercept on the sugar axis, at $\log t = \text{zero}$) and s is the amount formed during the second hour of diastasis (the slope of the curve multiplied by $\log 2$). These constants have been tentatively termed the primary and secondary saccharogenic values, respectively. It is probable that the diastase content of the flour is a considerable factor in determining the value of p, while the susceptibility of the native starch largely influences the value of s. The initial content i is conveniently included in the value of p. The simplicity of this expression arises from the fact that pH and T in the general expression have been kept constant and thus removed as factors. The complicated effect of pH is roughly indicated in Figure 5. This factor has also been discussed by Sörensen (1924), Rumsey (1922), Grewe and Bailey (1927), and Malloch (1929). This equation may be solved and the sugar level at any time conveniently determined from a monograph or a chart such as Figure 4. Some typical saccharogenic values are given in Figure 6. It is apparent that the results of other investigators may also be expressed by the logarithmic function. According to Collatz (1922), this was first indicated by the results of Heyl (1912), although they were obtained on soluble starch. While s has been termed the secondary value, it is by no means unimportant and in the case of certain flours may even be responsible for the production of more sugar in normal fermentation times than the primary value. For example, the primary and secondary values of certain mill streams from a Southwestern wheat are given in Table I.

TABLE I

SACCHAROGENIC VALUES OF CERTAIN MILL STREAMS DETERMINED BY FERMENTATION METHOD *b*

Stream	Primary, p % sugar	Secondary, s % sugar
1st break	1.94	.62
2nd break	2.14	.50
3rd break	3.60	.46
4th break	2.36	.56
Germ flour	5.76	.60
Low grade flour	3.56	.78

Sugar Level in Fermenting Doughs

The diastatic level,[6] S_d, in some fermenting doughs has been determined by adding the sugars fermented, as calculated from the expansion

[6] Diastatic level is here defined as the theoretical concentration of sugars at any time in a dough or suspension containing no added sugars *if* the fermentation rate were zero. It is essentially the potential sugar level decreased by the amount of sugar added in the formula.

produced, to the actual sugar level as determined by methods 3 and *2a*, and subtracting the amount of sugars added in the formula. While it is realized that there may be a slight error involved in this calculation due to the complexity of the zymase action, it nevertheless represents an

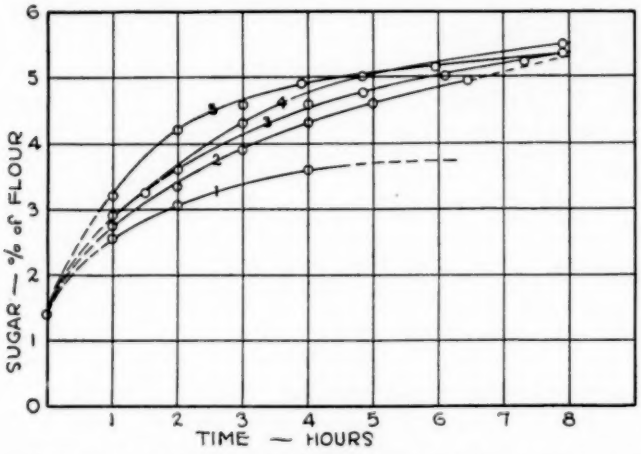

Fig. 7. Diastatic level in fermenting doughs. Northwestern hard patent flour. (1) Yeast-free dough. (2) 1% yeast dough. (3) 2% yeast dough. (4) 3% yeast dough. (5) Method *2b* unbuffered.

observed phenomenon, one which exists in actual practice. These results are illustrated in Figure 7. It is apparent that for a certain period of time, between one and three to four hours, the sugar concentration is very nearly a linear function of the time. Thereafter, the logarithmic phase is resumed. As has been shown by Rumsey (1922), Halton and Fisher (1932, 1932a), and others, the rate of change of acidity in the fermenting dough is most rapid during the initial period of fermentation. It is believed that this linear phase of the saccharogenic activity in fermenting doughs may eventually be explained by the compensating increase in enzymic activity with decrease in pH (see Grewe and Bailey, 1927).

Summary

1. Nearly ninety years after the discovery of enzyme action diastatic activity was recognized as an important factor in baking chemistry by Wood (1907) and contemporaries. Rumsey (1922) provided an analytical index of *autolytic* diastatic activity, by the use of which other investigators (Collatz and Racke (1925), Mangels (1926), Pascoe, Gortner, and Sherwood (1930)) showed that diastatic activity is an environmental factor, unrelated to flour strength. Since 1907 it has been frequently recognized that diastatic activity in dough should be

under the control of the baking technologist.[7] Requirements for data to enable the calculation of such control necessitate the development of adequate analytical methods.

2. Chemical methods for analysis of fermenting doughs for total sugars are at present inadequate. Some fermentation methods are described which are believed to be suitable for the present requirements of baking chemistry.

3. The potential sugar concentration in buffered suspensions and yeast-free doughs at constant temperature may be expressed by the equation:

$$S_p = \frac{s}{\log 2} \log t + p.$$

4. The potential sugar level in certain fermenting doughs and suspensions is shown to be higher than that in yeast-free doughs and to consist of a linear and a logarithmic phase.

Acknowledgment

The author wishes to express his appreciation for the direction of Dr. Charles N. Frey in this work.

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[7] Compare Frey (1933).

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PHYSICO-CHEMICAL STUDIES ON PROTEINS. VIII. THE ROTATORY DISPERSION OF THREE GLIADIN PREPARATIONS PEPTIZED BY DIFFERENT SOLUTIONS [1]

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Introduction

The generally accepted classification of the simple proteins is based largely on physical properties—the peptizability (solubility) of the proteins in various solvents. It has been shown, however, (Gortner, Hoffman and Sinclair, 1928, 1928a, 1928b, and Staker and Gortner, 1931) that protein preparations which are supposedly chemical entities show differences in peptizability much greater than might be expected from the nature of the peptizing solutions. Staker and Gortner found that half-molar solutions gave the following average peptizations (based on total nitrogen) of the proteins of *Triticum vulgare:* KF 29.79%, K_2SO_4 31.61%, KCl 36.88%, KBr 46.14% and KI 57.02%—a typical lyotropic series.

Sinclair and Gortner (1933) made a study of the peptization behavior of gliadin preparations. Three successive treatments of 100 gms. of gliadin each with a liter of molar potassium iodide solution extracted approximately 60% of the total nitrogen. The insoluble residue left was repeatedly dissolved in dilute alcohol and precipitated from water, and finally reprecipitated from absolute alcohol-ether and dried at 50° C. When again extracted with molar potassium iodide solution, four successive treatments peptized 57.40% of this protein which before was " insoluble " in the same solution. A " potassium bromide insoluble " fraction was obtained and " purified " in a similar manner. Treatment with molar potassium bromide solution peptized 20.84% of the previously " insoluble " gliadin. Molar potassium chloride solutions showed a comparable behavior, although the absolute " solubilities " were less. These authors showed further that the total nitrogen content and the

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free amino nitrogen was constant in each fraction. The Hausmann analyses of the different preparations and fractions were in good agreement. Sinclair and Gortner (1933) conclude that the difference in "solubility" is *accounted for by a physical difference in the degree of micellar aggregation and not to a chemical difference such as would accompany a mixture of protein varieties.*

Haugaard and Johnson (1930), also working with gliadin, obtained their fractions by cooling alcohol sols and removing the precipitated gliadin. They report small differences in tyrosine and tryptophan content and in the rate of pepsin hydrolysis of the fractions. Their work has already been considered by Sinclair and Gortner (1933) who do not believe that the evidence presented warrants the belief that gliadin is a mixture of different proteins.

The Problem

The gliadin fractions studied by Sinclair and Gortner (1933) were available for further study. Optical rotatory power is a valuable criterion of chemical identity or non-identity, and it was believed that it might be a useful test in this case. If the fractions are chemically different we would expect to find differences in the optical behavior, but this would not necessarily follow. On the other hand, if differences were found, this would conclusively indicate chemical differences in the fractions.

The rotatory dispersion, *i.e.,* the variation in optical rotation with light frequency, gives a more complete picture than do observations made with only one spectral band, such as the D line of the sodium spectrum. It may happen that the optical activities would coincide or closely approach each other at one frequency, but be distinctly different at another either lower or higher.

In this study, therefore, it was desired to approach the problem of the differential peptization of gliadin in the following ways:

1. To study the effect of different peptizing agents on the rotatory dispersion,

2. To determine whether the method of preparation of the gliadin affects the rotatory dispersion, and

3. To attempt to discover if there is a demonstrable Hofmeister series with respect to the rotatory dispersion.

Historical

Optical rotatory power has been used very widely to characterize different substances. It is used in the identification of sugars, in studying the position of substituted groups in organic syntheses, and in studying purity of materials and rates of reaction.

The rotatory dispersion gives additional information; the rotations may coincide at one point, but differ if other portions of the spectrum are used as a light source. Drude's equation describes this variation with light of different wave lengths. Drude's equation

$$[a] = \frac{k_1}{\lambda^2 - \lambda_1^2} \pm \frac{k_2}{\lambda^2 - \lambda_2^2} \pm \frac{k_3}{\lambda^2 - \lambda_3^2} \pm \cdots$$

can be reduced to the general equation

$$[a] = \Sigma \frac{K_n}{\lambda^2 - \lambda_n^2}$$

which expresses the summation of the vibrations of the different electrons which give rise to absorption and dispersion.

The rotary dispersion of many substances (those which show "simple rotatory dispersion" and which have only a single major absorption band, λ_o) may be expressed by a single term of the Drude equation

$$[a] = \frac{k_o}{\lambda^2 - \lambda_o^2}$$

where $[a]$ = specific rotation at wave length λ
λ_o = wave length of dominant absorption of the substances
k_o = a constant characteristic of the substance

For a detailed discussion of the mechanics and theory of optical rotation and rotatory dispersion, the papers of the Faraday Society (1914, 1930) offer excellent reviews.

The Optical Rotatory Power of Gliadin. The proteins in general exhibit levo-rotation. Hewitt (1927) gives the following data for certain animal proteins:

Protein	$[a]$			
	λ = 4359 Å	λ = 5461 Å	λ = 5780 Å	λ = 6660 Å
	Degrees	*Degrees*	*Degrees*	*Degrees*
Serum albumin	−151.5	−78.1	−67.0	−47.3
Serum pseudo-globulin	−118.2	−68.7	−59.8	−43.1
Serum euglobulin	−105.2	−61.2	−54.0	−39.4
Egg albumin	− 83.9	−44.5	−38.3	−27.5
Lactalbumin	− 88.9	−47.5	−41.1	−28.6
Casein	−184.4	−105.1	−91.2	−66.0
Gelatin	−496.8	−282.8	−248.2	−181.1

Gliadin likewise is levo-rotatory. Kjeldahl (1896) in the period from 1891 to 1895 collected a number of samples of wheat from all parts of the world. He reports the specific rotation of the alcohol soluble protein from these as −92°. His gliadin preparations dissolved in glacial acetic acid gave $[a]_D = -81°$, in 1 to 5% acetic acid, −111°, and a similar value was found if weak alkalis were used for peptization.

Osborne and Harris (1903) noted a difference in the specific rotation with the length of observation tube used. For gliadin preparations of similar concentration, in a 20 cm. tube, $[a] = -91.9°$, and in a 10 cm. tube, $[a] = -92.55°$.

The rotatory power in a number of organic solvents was later determined by Mathewson (1906).

Greaves (1911) determined the rotation of gliadin in different percentages of alcohol. He also determined the effect of temperature on the rotatory power and reported that the rotation at 10° C. is 0.15° greater than at 60° C. From the variations which he found due to the effect of alcohol concentration, it would appear that the temperature effect is much less than the experimental error.

In studying the factors affecting flour strength Woodman (1922) found the rotation of gliadin in 70% alcohol to be —93.6° for Manitoba flour and —93.78° for English flour. He and Kjeldahl concur in reporting the rotation of preparations in weak alkalis as —111°.

As far as can be ascertained, Dingwall (1924) was the first to determine the rotation of gliadin with light of more than one wave number. The average of three preparations in 70% alcohol give the following values at 21° C.:

λ in mμ	[α]
650	−72.4°
589	−92.2°
530	−119.9°
480	−151.4°

Haugaard and Johnson (1930) in a paper received after the present study was well under way, report the following values:

λ in mμ	[α]
666	−90.8°
578	−95.9°
546.1	−110.2°
435.4	−200.5°

These workers state that by fractional precipitation, accomplished by cooling the alcohol solution, they obtain fractions in which $[\alpha]_{578}^{20°}$ shows differences "which are too large to be due exclusively to experimental error." They consider that the rotation varies with the "maximum solubility." The products of acid hydrolysis showed no large differences.

In surveying the literature no mention was found of the rotatory dispersion of gliadin peptized by different inorganic salt solutions or of gliadins prepared by different technics.

Experimental

As was indicated in the introduction, it was planned to measure the rotatory dispersion of gliadin prepared by different methods and peptized by different solutions, namely by normal solutions of KI, KBr, KCl, NaCl and LiCl and also by water, 10% and 70% ethyl alcohol.

THE MATERIALS. Three gliadin samples were used. These were the ones studied by Sinclair and Gortner (1933) and designated by them gliadin "A" (alcohol method), gliadin "B" (acetic acid method of Blish and Sandstedt, 1926), and gliadin "D" (electrodyalized acetic acid gliadin).

THE APPARATUS. The equipment necessary for the determination of rotatory dispersion consists of a light source, a device for removing from this light all but a narrow spectral band, and, for the actual observations, a polariscope. The apparatus used was as follows:

The Light Source. In order to obtain a brilliant, constant light which would give a sufficient intensity for polarimetry in any narrow spectral band, an incandescent tungsten ribbon filament was used. This ribbon is about 2×30 mm. and is mounted vertically in an evacuated bulb. It operates on 5 to 6 volts, A. C., the current coming through a transformer, in the primary of which a rheostat is used to control the voltage of the secondary. The lamp is placed in a ventilated housing and is cooled by forced circulation of air.

The ribbon filament provides a light source of constant intensity and has the additional advantage of producing an even field when viewed through the polariscope. It produces a continuous spectrum and gave satisfactory intensities at all wave bands used except in the blue where gliadin shows high absorption.

The Monochromator. A Bausch and Lomb Quartz Monochromator (described in detail by Kurtz, 1926) was used between the light source and the polariscope. The instrument was calibrated against six spectral lines of the following wave lengths (with equivalent frequencies):

Source	λ in mμ	ν (frequency)
Sodium flame	589.3 (ave.)	5.089×10^{14}
Lithium flame	670.8	4.469
Mercury arc	623.4	4.811
" "	546.1	5.491
" "	491.6	6.100
" "	435.8	6.881

All of these except the last were used for the polarizations of the gliadin solutions.

The Polariscope. There was available a Schmidt and Haensch half-shadow polariscope with Lippich polarizer, reading to 0.01 angular degrees. It was adjustable for zero reading, half-shadow angle and for varying focus of the eye-piece on the polarizing prism. The observation tubes were set in a sliding rack so arranged that the empty (zero) tube could be thrown out and the unknown thrown in almost instantaneously.

USE OF THE APPARATUS. The light source was kept as bright as

was compatible with a fair length of life of the bulb. It seemed preferable to limit the intensity of light reaching the polariscope by narrowing the slits of the monochromator to the desired point. In this way it was possible to use a narrow wave band. Slits of 0.05 to 0.2 mm. were used. The actual width of the wave band was not determined.

THE METHOD. A weighed amount of gliadin, 1.0 or 2.5 gm. depending on the solubility, was introduced into a 200 cc. bottle and 50 cc. of the peptizing solution was added, meanwhile turning the bottle so as to distribute the wetted protein about the surface of the bottle. The bottle was mounted horizontally on a vertically rotating arm, so that the peptizing medium was constantly flowing over the gliadin on the walls of the bottle. This method of stirring was used to avoid the foaming and consequent loss of protein incident to rapid stirring or shaking.

The mixture was rotated for seven hours, let stand overnight and rotated for another hour. The liquid was then decanted and filtered on a Büchner funnel through two S. and S. No. 595 filter papers. This filtrate was not always sufficiently clear to use for rotation measurements, and in these cases the preparation was allowed to stand at 15° C. for a time to permit the system to come to equilibrium, either by complete solation or by precipitation of the suspended protein. It was found that heating or cooling had no demonstrable effect on subsequent measurements of rotatory dispersion (in these cases considerable time was allowed for the system to come to equilibrium at room temperature), nor did the interval between the peptization and observation seem to affect the rotation.

The measurements of optical rotatory power were made at 25° ± 1° C. Observation tubes were chosen which would give the greatest actual rotation without obscuring the field by the turbidity of the solution; 1 dm., 2 dm. and 3 dm. tubes were used. In most cases readings were taken until three or more agreed within 0.02° observed rotation.

It was observed that the most closely agreeing readings were obtained when the light input was reduced to the point where the illumination of the field was dim. Gliadin absorbs light of the higher frequencies to a much greater degree, and to compensate for this it was necessary to open the slits of the monochromator wider for measurements in the blue end of the spectrum.

Nitrogen was determined by the micro-Kjeldahl method, employing direct Nesslerization, and calculated to gliadin by the factor 5.7.

The rotation is expressed as specific rotation,—the rotation which would be produced by one decimeter of the solution, containing one gram of the active substance per cubic centimeter.

ERRORS OF MEASUREMENT. The observed rotation may vary with a number of factors, including the following:

(a) *The setting of the monochromator.* The optical activity is an exponential function of the light frequency, hence an error in setting the monochromator introduces an error in the observation. Although the instrument cannot be adjusted with absolute accuracy, the error from this source is negligible. The construction of the wave-length drum is such that more accurate settings are possible at the higher frequencies, at which the greater errors might otherwise occur.

(b) *The temperature.* Greaves (1911) has shown that the temperature coefficient for the optical rotation of gliadin is of the order of 0.05° per change of 10° C. The small differences of temperature in the present study are of slight importance.

(c) *Differences in concentration and turbidity.* Turbidity appeared to be the cause of the greatest error in readings. This may be ascribed to two factors—the actual lack of sharpness of reading due to a hazy field or to lack of sufficient illumination as encountered in the deep red and especially in the blue where absorption is high. The observations at the sodium D line are probably the most accurate.

Miller and Andrews (1930) call attention to the fact that a measurable difference may result from turbidity, even though the fields are not hazy. They report a considerable difference in specific rotation when measurements of the same solution are made in tubes of different lengths. They consider it advisable to use tubes of different lengths and to take the mean of these readings. This was not done in the present case, since it was necessary to use the longest possible tube in order to obtain an absolute rotation greater than 0.5 to 1.0°.

The correction for concentration such as we apply in calculating the specific rotation of sugars, *e.g.* $[\alpha]_D$ for sucrose equals $66.412 + 0.012673\ c - 0.0003766\ c^2$, is unknown for gliadin and, from the determinations of Haugaard and Johnson (1930) negligible.

(d) *Solvent.* The literature is abundantly supplied with reports of the effect of solvent on the rotatory power. The changes are variously ascribed to dissociation, hydration or solvation, shift in isomeric equilibrium, sol-gel transformation, etc.

Lucas (1930) has classified the effects of solvents in three groups:

(1) The active substance and the solvent may interact in accordance with their chemical properties,

(2) The active substance, on dissolution, shows the phenomenon of mutarotation,

(3) The rotatory power of the active substance varies with the solvent, but without any detectable mutarotation or any obvious chemical action between solvent and solute.

It must be recognized that this last case is extremely common and, indeed, that active substances whose rotatory power is practically independent of concentration and solvent are very rare.

Patterson (1914) has discussed some relations between temperature, solvent and wave length and their effects on rotation.

(e) *Nesslerization.* The colorimetric comparison on the digested and Nesslerized solution with the standard is probably exact to two per cent.

Data

The summarized data [2] are given in Tables I, II, and III, and Figures 1 and 2. The character of the light used is expressed in terms of oscil-

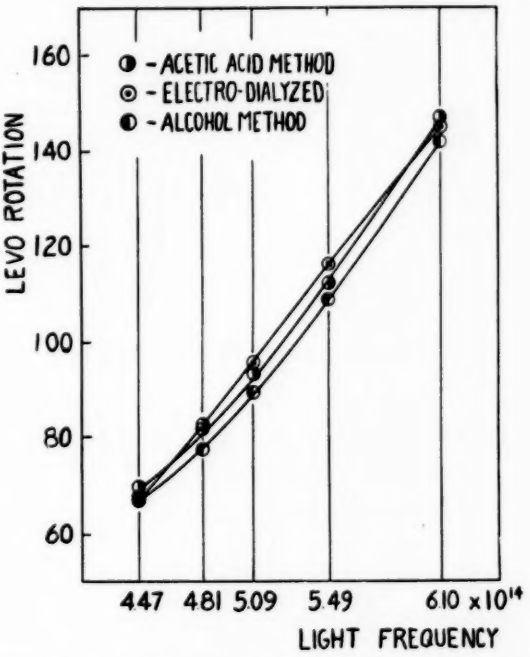

Fig. 1. Curves for the average rotatory dispersion for the three gliadin preparations.

lation frequency (ν). In Figure 1 are shown the rotary dispersion curves for the three samples of gliadin which were studied. Figure 2 shows our average data for the three samples together with the values for the preparations studied by Dingwall (1924) and those studied by Haugaard and Johnson (1930). Haugaard and Johnson report a rotation of $-90.8°$ at 666.0 mμ. When we drew the curves it was evident that this was a typographical error in their paper and this was confirmed by a personal communication with Dr. Haugaard, who replied that the correct value was 70.8°. This value is accordingly plotted on the curve.

[2] The complete data are all tabulated, together with other graphs in a manuscript thesis entitled "The Rotatory Dispersion of Gliadin Peptized by Different Solutions" by H. O. Wiles, filed in the Library of the University of Minnesota.

TABLE I

THE ROTATORY DISPERSION OF GLIADIN PREPARATIONS B, D, AND A, WITH RESPECT TO THE PEPTIZING SOLUTION

ν	Peptized by 10% EtOH		Peptized by 70% EtOH			Peptized by H_2O		
	B	D[1]	B	D[1]	A	B[1]	D[1]	A[1]
	Degrees	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*
4.47×10^{14}	−70.8	−60.2	−74.7	−67.9	−76.6	−64.8	−72.5	−60.7
4.81	−81.1	−80.0	−85.8	−81.2	−89.2	−80.1	−84.3	−80.1
5.09	−92.2	−96.2	−96.7	−93.4	−97.5	−91.6	−98.0	−89.3
5.49	−111.8	−113.1	−119.9	−110.3	−116.0	−106.8	−125.2	−105.0
6.10	−140.8	−147.9	−149.9	−144.4	−144.1	−141.4	−147.2	−142.3
Concentration: gm. gliadin/100 cc.	5.03	1.215[2]	4.36	1.68[2]	1.67	2.085[2]	0.385[2]	0.215[2]

ν	Peptized by *N* KI			Peptized by *N* KBr		
	B	D[1]	A	B[3]	D	A
	Degrees	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*
4.47×10^{14}	−74.5	−72.3	−73.5	−60.2	−56.6[3]	−33.4[3]
4.81	−82.2	−82.2	−79.1	−71.1	−81.6	−57.8
5.09	−93.4	−94.8	−89.7	−98.5	−95.8	−80.0
5.49	−114.7	−110.4	−110.0	−147.5	−116.8	−108.9
6.10	−161.0	−144.6	−138.6	−142.0	−133.0[3]	−171.0[3]
Concentration: gm. gliadin/100 cc.	0.90	2.665	1.38	0.08	0.43	0.15

[1] Average of two complete series.
[2] Average concentration.
[3] Not included in averages (Table III) because of low concentration of the solution and errors in reading.

TABLE II

ROTATORY DISPERSION OF GLIADIN D IN HALF-MOLAR SOLUTIONS

ν	Peptizing Solution					
	5% EtOH	$\frac{N}{2}$ KI	$\frac{N}{2}$ KBr	$\frac{N}{2}$ NaCl	$\frac{N}{2}$ LiCl	Ave.
	Degrees	*Degrees*	*Degrees*	*Degrees*	*Degrees*	*Degrees*
4.47×10^{14}	−55.2	−71.8	−66.0	−62.2	−61.9	−63.42
4.81	−73.5	−79.8	−73.5	−66.2	−76.5	−73.90
5.09	−84.0	−89.5	−75.5	−73.3	−94.6	−83.38
5.49	−105.0	−104.4	−90.5	−93.3	−113.0	−101.24
6.10	−160.0	−140.1	−122.4	−121.3	−134.8	−135.72
Concentration: gm. gliadin/100 cc.	0.38	1.10	0.32	0.81	0.09	

TABLE III

AVERAGE ROTATORY DISPERSIONS OF GLIADIN PREPARATIONS B, D, AND A

ν	Gliadin B	Gliadin D	Gliadin A	Average
	Degrees	*Degrees*	*Degrees*	*Degrees*
4.47×10^{14}	−69.90	−68.19	−67.88	−68.66
4.81	−82.50	−81.87	−77.24	−80.54
5.09	−93.08	−95.58	−89.14	−92.60
5.49	−112.00	−114.95	−108.98	−111.98
6.10	−146.90	−145.83	−141.83	−144.85

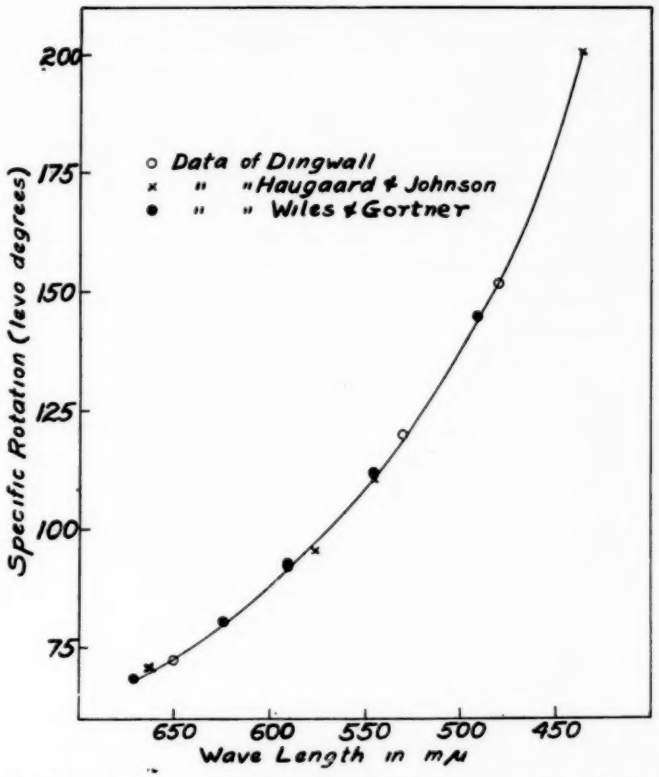

Fig. 2. Curve for the average rotatory dispersion of gliadin from all available data.

Discussion

Gliadin B shows little or no significant variation in [α] for different peptizations except for the KBr series. This series is regarded as unreliable since the readings show no uniform variation and are inconsistent in the higher wave numbers. This material was prepared by the acetic acid method and some of the acid was still held by the protein (Sinclair and Gortner, 1933). Since acetic acid is an excellent peptizing agent, it is probable that there was a joint peptizing action by the acid and by the added solution. Solutions of gliadin B peptized by potassium, sodium and lithium chlorides were too dilute to give measurable readings in the polariscope. Potassium sulfate gave no appreciable peptization.

Gliadin D is electrodialyzed gliadin B. The optical rotations and dispersions are uniform except for the peptization by water which tends to be somewhat high. The sols peptized by normal potassium bromide give more reliable readings than was the case with gliadin B, except for

the readings in the blue and deep red. These readings were hazy and are regarded as unreliable. As before, sols in normal solutions of KCl, NaCl, and LiCl gave readings too small to be considered.

Gliadin A, prepared by the alcohol method, gave less uniform readings. The water peptization in this case conformed to the accepted value for gliadin, but the 70% alcohol preparations gave high values in every case. It was difficult to obtain clear solutions of this preparation, which fact probably accounts in part for the high readings. As before, the values for the *N* KBr peptizations are unreliable at the spectral extremes and are only approximate at the other frequencies. The alkali chlorides and potassium sulfate failed to peptize enough to give a readable preparation.

The peptizations with half-normal solutions gave disappointing results. With the exception of N/2 KI the sols were dilute and the errors of measurement too great to give reliable readings. It would appear from the behavior of this series that the peptization in half-normal solution is predominantly due to the water. It is evident that there is too much erratic variation to permit any conclusions to be drawn as to a lyotropic series with respect to rotatory dispersion.

On comparing the averaged results of each preparation, we find little evidence of difference in rotatory dispersion between the different preparations. It will be noted that the values for gliadin A tend to be a little lower than those for gliadins B and D. It is believed that these lower values are attributable to other causes than a difference in the gliadin composition. In general the very dilute preparations gave low readings, moreover some of the more concentrated preparations of gliadin A were slightly turbid, thus contributing to inexact readings.

Hewitt (1927) as well as Haugaard and Johnson (1930) have shown that the rotatory dispersion of several proteins fulfills the criterion of the one-term equation for simple rotatory dispersion,

$$[\alpha]_\lambda = \frac{k_0}{\lambda^2 - \lambda_0^2}$$

and that the values of $1/\alpha$ plotted against λ^2 should give a straight line. The averaged values have been plotted in Figure 3 and it is interesting to note that the readings with the three most reliable wave bands give practically a straight line. The graph also indicates that, in general, the observed readings in the deep red were too high and in the blue too low.

Summary

Gliadin prepared by three methods has been peptized by 10% and 70% ethyl alcohol, distilled water, *N* KI and *N* KBr solutions. The

optical rotatory power of these sols has been determined at five light frequencies with the following average results:

ν	$[\alpha]^{25}$
4.47×10^{14}	$-68.66°$
4.81 "	$-80.54°$
5.09 "	$-92.60°$
5.49 "	$-111.98°$
6.10 "	$-144.85°$

There was no difference in the rotatory dispersions of the three protein preparations which we regard as greater than errors incident to the determinations.

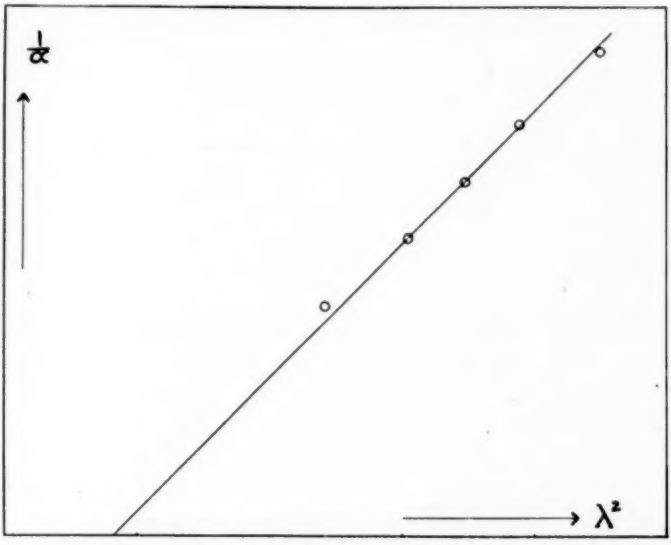

Fig. 3. Showing the straight line relationship for $1/[\alpha]$ and λ^2 for gliadin solutions.

Normal solutions of potassium chloride, sodium chloride, lithium chloride and potassium sulfate failed to peptize enough gliadin to yield a readable optical rotation.

The peptizing agent seems to have no demonstrable effect on the rotatory dispersion; if there is a lyotropic series effect it is less than the errors of the experimental method used.

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SOME EXPERIENCES WITH THE WHEAT MEAL FERMENTATION TIME TESTS FOR EVALUATING SOFT WHEATS [1]

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(Read at the Convention, June, 1933)

"The Wheat-Meal Fermentation Time Test" is a short method for determining the gluten strength of wheat-meal. It is being used by a few investigators for measuring the probable strength of the flour that will be obtained from milling samples of wheat. It is of special interest to the plant breeder who, before he has grown a sufficient amount of material from which to mill and bake, needs to know something about the quality of the different strains of wheat which he is developing. Any method which will give information on the quality of a wheat without milling the sample will likewise be more than welcomed by the cereal chemist.

This test is a modification of the one originated by Saunders and Humphries (1928) for testing the gluten strength of flours. It has also been used by Pelshenke (1930, 1933), and by Cutler and Worzella (1931, 1933). The latter use the test in their plant breeding work for the selection of strains of wheat. They report a high positive correlation between the test and protein content of flour, loaf volume, absorption, and vitreous kernels. From their work on samples obtained from different millers they report a high positive correlation between the test and the quality of wheat desired for making a flour for particular purposes. The wheats preferred for bread flour have a long "time" test, and those for pastry flour a short "time" test.

This paper gives some of the experiences of the Chemical Division of the Michigan Agricultural Experiment Station with the "time" test and some results obtained in comparing it with three other tests which have been used by the station for evaluating Michigan wheats, *viz.*, the protein content of the flour, the volume of the baked loaf, and the volume of the expansion of the dough as determined by Wilsie, Robinson and Winter (1932). It also includes some preliminary work on a modi-

[1] Published with the permission of the Director of the Experiment Station as Journal Article 176 (n.s.). Contribution from the Chemistry and Farm Crops Sections.

[2] Research Associate in Chemistry.

[3] Graduate Assistant in Farm Crops. The data presented are in partial fulfillment of the degree of Master of Science.

TABLE I

COMPARISON OF THE FOUR METHODS FOR THE DETERMINATION OF QUALITY IN WHEAT

Crop year			1931					1932			
Wheat variety	Sample No.	Location	Protein	Loaf volume	Expansion	Time	Location	Protein	Loaf volume	Expansion	Time
			P.ct.	*Cc.*	*Cc.*	*Min.*		*P.ct.*	*Cc.*	*Cc.*	*Min.*
American Banner	1	M. S. C.	11.17	526	845	27	M. S. C.	—	—	—	—
	2	Augusta	11.40	546	920	28	Coldwater	9.18	508	905	28
	3	Lake City	13.42	588	850	38	Lake City	—	—	—	—
	4	Monroe	9.58	536	880	29	Monroe	9.19	544	845	28
	5	Eagle	12.16	551	890	25	Marlette	10.55	525	951	30
	6	Lake Odessa	12.11	494	820	27	Jasper	10.20	528	952	25
	Mean		11.64	538	867	29					
Red Rock	7	M. S. C.	10.70	608	980	169	M. S. C.	9.44	606	992	160
	8	Augusta	11.01	602	1000	144	Coldwater	10.13	603	1076	138
	9	Lake City	14.57	606	935	157	Lake City	9.42	591	1081	156
	10	Monroe	9.88	572	1015	150	Monroe	9.43	593	983	133
	11	Eagle	14.34	578	935	166	Marlette	11.82	617	1048	125
	12	Lake Odessa	11.61	612	1015	140	Jasper	—	—	—	—
	Mean		12.02	596	980	154		10.05	602	1036	142
Bald Rock	13	M. S. C.	12.56	534	935	132	M. S. C.	9.68	528	936	146
	14	Augusta	12.04	562	970	127	Coldwater	9.66	529	848	109
	15	Lake City	13.86	554	950	112	Lake City	9.59	534	1009	148
	16	Monroe	10.21	534	865	122	Monroe	10.29	540	905	120
	17	Eagle	12.79	596	1030	154	Marlette	10.93	539	903	113
	18	Lake Odessa	12.38	582	945	151	Jasper	11.75	526	902	123
	Mean		12.31	560	949	133		10.32	533	917	127

TABLE I—*Continued*

Crop year			1931					1932			
Wheat variety	Sample No.	Location	Protein	Loaf volume	Expansion	Time	Location	Protein	Loaf volume	Expansion	Time
			P.ct.	*Cc.*	*Cc.*	*Min.*		*P.ct.*	*Cc.*	*Cc.*	*Min.*
No. 912203 [1]	19	M. S. C.	10.78	572	975	124	M. S. C.	9.96	508	855	34
	20	Augusta	11.79	625	1080	89	Coldwater	10.24	507	875	103
	21	Lake City	14.32	636	1105	64	Lake City	9.91	553	926	151
	22	Monroe	10.31	602	990	135	Monroe	10.79	535	894	99
	23	Eagle	13.28	636	1140	110	Marlette	10.40	510	942	91
	24	Lake Odessa	12.26	560	985	80	Jasper	11.06	593	952	65
	Mean		12.12	604	1046	100		10.56	534	905	91
Berkeley Rock	25	M. S. C.	12.07	554	990	154	M. S. C.	10.32	600	1008	142
	26	Augusta	12.11	560	1050	175	Coldwater	10.06	543	963	129
	27	Lake City	14.63	572	1000	169	Lake City	9.82	597	1127	143
	28	Monroe	10.50	540	910	136	Monroe	10.34	575	994	118
	29	Eagle	13.79	593	1035	176	Marlette	12.71	554	1074	48 [2]
	30	Lake Odessa	12.74	598	1030	141	Jasper	11.50	596	1080	118
	Mean		12.64	570	1002	157		10.79	578	1041	138

[1] The authors have no explanation for the variations in this variety.
[2] Omitted from the mean.

fication of the "time" test whereby the "volume" of the water displaced by the expanding dough ball is measured instead of the "time" of the fermentation.

The technique of the method is similar to that used by Cutler and Worzella (1931) and is as follows:

Formula—Wheat meal .....10 gms.
Yeast suspension (10%) in distilled water.....5.5 cc.
Distilled water .....according to absorption

Procedure—The meal is prepared by grinding the sample of grain in a Wiley mill to such a fineness that it will pass through a sieve with 1 mm. circular openings. The yeast suspension is prepared and allowed to stand in the fermentation cabinet at a temperature of 30° C. for 30 minutes before using. The dough ball is prepared by mixing the meal with a porcelain spatula in a small evaporating dish until it holds together. It is then folded together into a compact mass, taken in the hands and rolled into a ball which is placed in the cabinet in a 150 cc. beaker containing approximately 80 cc. of distilled water. At first the dough ball sinks to the bottom but later it rises to the top, slowly spreads out, and finally disintegrates and fragments of it fall to the bottom of the beaker. The difference in minutes between the time the dough ball is placed in the water and the time of the first fall of dough [4] is taken as the "time" of the test.

Determinations were made by each of the four tests mentioned above on the following five varieties of wheat: American Banner, Red Rock, Bald Rock, No. 912203, and Berkeley Rock. Each of these varieties was grown during 1931 and 1932 at six different locations in Michigan. A comparison of the data obtained by these four methods is found in Table I which gives the averages of duplicate determinations (a third determination is included where the results did not check reasonably close) and the varietal mean for each year.

The data in Table I indicate (1) that the results by the wheat-meal fermentation time test show proportionally greater differences between individual samples and also between variety means than do those of any other of the tests; (2) that there is no direct relationship between the time test and the other tests. However, in all of the varieties except No. 912203 there is a general relationship between the time and the volume of loaf and also between the time and the expansion of the dough. For example, if the volume of the loaf or the expansion of the dough is large the time is long, and vice versa; and (3) that the differences in the "time" between widely different varieties such as American Banner and Red Rock, is much greater than would be anticipated from the differences shown in any of the other tests.

Applying statistical methods and considering any difference which is greater than 3.3 times the probable error as significant, the number of significant differences between the five varieties as indicated by the four methods is shown in Table II.

[4] Although the time when the first fall of dough occurs is generally easily recognized especially in the cases of the softer flours, there are some samples in which this is not true.

TABLE II

NUMBER OF SIGNIFICANT DIFFERENCES BETWEEN VARIETIES

Year	Protein in wheat	Volume of loaf	Expansion of dough	W.M.F.T.[1]	Possible number
1931	0	5	5	9	10
1932	0	6	6	4	10
Total	0	11	11	13	20

[1] Wheat-meal fermentation time.

The results in Table II show a greater number of significant differences between varieties by the "time" test than by any of the others. It is interesting to note that in 1931 there were nine significant differences out of a possible ten. In 1932, however, only four differences were significant out of ten.

Again applying statistical methods, the correlation between any two of the four methods used, is shown in Table III.

TABLE III

COEFFICIENTS OF CORRELATION BETWEEN ANY TWO OF THE FOUR METHODS USED FOR THE DETERMINATION OF QUALITY IN WHEATS

	Year	Protein in wheat	Volume of loaf	Expansion of dough	W.M.F.T.[1]
Protein in wheat	1931	———	.44±.09	.33±.11	.22±.07
Volume of loaf		.44±.09	———	.82±.03	.66±.06
Expansion of dough		.33±.11	.82±.03	———	.64±.07
W.M.F.T.[1]		.22±.07	.66±.06	.64±.07	———
Protein in wheat	1932	———	.48±.07	.51±.07	.41±.08
Volume of loaf		.48±.07	———	.86±.08	.78±.04
Expansion of dough		.51±.07	.86±.08	———	.69±.05
W.M.F.T.[1]		.41±.08	.78±.04	.69±.05	———

[1] Wheat-meal fermentation time.

From the above results, it will be seen that the highest positive correlations are found to exist between volume of loaf and expansion of the dough. The second and third highest correlations are between the wheat-meal fermentation time and (*a*) the volume of loaf and (*b*) the expansion of the dough.

In making determinations by the wheat-meal fermentation time test, the procedure required the constant attention of the operator in order to record the exact time at which the dough ball began to disintegrate. In trying to overcome this difficulty an apparatus was set up by which the water displaced by the expansion of the dough ball could be measured. This consisted of a beaker and a 750 cc. wide-mouth glass bottle

with a one hole rubber stopper through which passed a bent glass tube as shown in Figure 1.

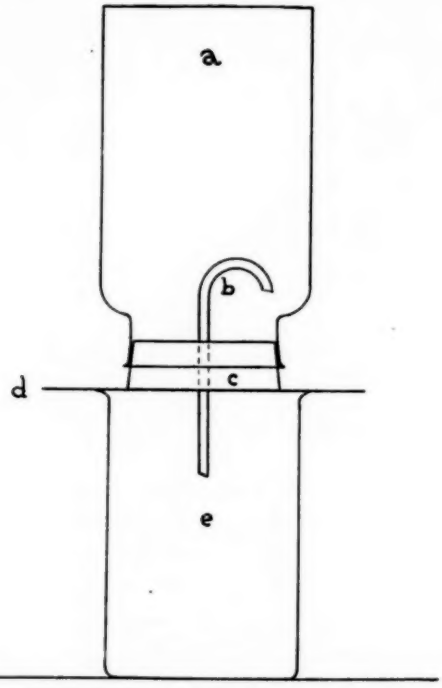

Fig. 1. Apparatus for automatic measurement of expansion when using wheat-meal fermentation test. *a*, bottle; *b*, glass tube; *c*, stopper; *d*, card board; *e*, beakers.

The technique using the above apparatus is as follows:

Formula—Wheat-meal .....30 gms.
Yeast suspension (10%) .....20 cc.
Water .....according to absorption

Procedure—Five hundred cc. of water is placed in the bottle, the dough ball dropped in[2] and the stopper inserted. The bottle is then allowed to stand until the dough ball rises to the top of the water when it is carefully inverted, the excess water allowed to run out and then it is placed over the beaker. As the dough ball expands the displaced water collects in the beaker. The number of cubic centimeters of water displaced is taken as the measure of the strength of the meal. After the bottle is inverted over the beaker it is unnecessary to give more attention to the apparatus until the disintegration of the dough ball is completed. Very little water is displaced after the first breaking up of the dough ball.

Table IV gives data which show triplicate determinations of the "time" in minutes by the above-mentioned time test, and duplicate determinations of the water displaced by the modified test. This table also gives the average volume of loaf from the corresponding wheats. These data are typical of average results obtained by the methods.

[2] A small aluminum cup containing barium hydroxide was placed in some of the bottles so that it floated on the surface of the water in order to absorb any liberated carbon dioxide. The increase in displacement when this was omitted was so small that this precaution was omitted. It is recognized that this method does not give absolute results. The data however are comparative.

The data in Table IV show that the time test and the modified test appear about equal in accuracy, that each shows a significant difference

TABLE IV

COMPARISON OF TIME WITH WATER DISPLACEMENT AND VOLUME OF LOAF

Sample No.	Time	Displacement	Volume of loaf
	Min.	*Cc.*	*Cc.*
1	28	47	508
	28	49	
	28		
2	28	51	522
	92	56	
	28		
3	29	49	525
	32	61	
	29		
4	24	43	528
	25	71	
	27		
5	—	130	540
	118	140	
	117		
6	29	45	544
	28	53	
	27		
7	161	167	591
	158	172	
	150		
8	137	183	593
	139	198	
	—		
9	133	158	603
	143	178	
	139		
10	157	165	606
	163	179	
	160		
11	143	147	608
	62	141	
	146		
12	100	152	617
	147	163	
	129		

between the harder and the softer wheats, and that the two methods rank about equal when compared with loaf volume. The advantages of the modification lie in the fact that one operator is able to make a

larger number of determinations in a given time, or, in the case of running a small number of samples, he will not need to give his entire attention to the samples, and that the larger amount of material is more easily handled. The disadvantage is that the larger amount of material may not be available.

From the data presented in this paper, one must conclude that the wheat-meal fermentation time test gives greater differences between samples of flour than do any of the other three tests which were studied. However, since these differences do not show a high positive correlation with either protein content or loaf volume which, up to the present, have generally been considered as the criteria for evaluating a flour, it is difficult to give an adequate interpretation of the results. Possibly the old idea of evaluating a flour by its protein content or the volume of the loaf should be discarded.

Summary

1. The wheat-meal fermentation time test was compared with three other tests (protein content of flour, volume of loaf, and expansion of dough) for determining quality of wheat.

2. The wheat-meal fermentation time test shows a fair positive correlation with volume of loaf and expansion of the dough, but not with protein content of flour.

3. This test shows proportionally greater differences between samples of flour than do any of the others. However, since there is only a fair correlation between the results by this test and the volume of loaf or the protein in the flour, no attempt has been made to interpret the results obtained by its use.

4. A modification of the test is described whereby the expansion of the dough ball is measured instead of the time of fermentation.

5. Preliminary results by the modified test compare favorably with those of the time test and the modification requires less of the operator's time.

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THE STANDARD BAKING TEST UNDER ENGLISH CONDITIONS

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(Read at the Convention, June, 1933)

Introduction

When I attended the American Association of Cereal Chemists' Convention in Minneapolis in 1928, I was one of those speakers who advocated giving the suggested standard baking test a fair and proper trial. I further stated that I would like to investigate it under European conditions. Unfortunately, there have been delays, partly due to equipment difficulties and partly due to the fact that my recent travels have interfered with the work at Dover, which I was anxious to do personally.

Perhaps it was just as well, as the test has, of course, been developing in the interval as experience has grown.

Let us examine what is the actual idea of the test. It is an attempt on the part of cereal chemists to evolve a simple laboratory baking procedure which will foretell the baking properties of a flour with a reasonable degree of precision, so that all chemists can, by adopting the specified standard method, be certain of arriving at the same evaluation of the baking qualities of a given flour. This is obviously an ambitious program and full success cannot be expected in a short time. However, the test has now been under intense investigation in hundreds of laboratories for over three years and, to say the least, the results appear to be a little confusing.

In the first place, the test has almost drifted away from the original specification, the only main standard left intact being the 100 gms. of flour used. Even at the commencement of this work Blish (1928) suggested that, besides the standard procedure of

100 gms. of flour on 15% moisture basis
3 gms. yeast (3%)
1 gm. salt (1%)
2.5 gms. sugar (2½%)
58% water absorption

there should be four additional and supplementary variations.

(*a*) Basic procedure but varied absorption.
(*b*) Basic procedure but varied fermentation time.
(*c*) Basic procedure plus addition of potassium bromate.
(*d*) Basic procedure but varied mixing method.

The necessity of introducing such variations itself queries the possibility or desirability of attempting to make standard baking tests, but since then many experimenters of note have introduced further variations. Besides, perhaps, the more excusable differences in mixing, moulding, etc., which one must expect in the early stages of such work, many workers prefer to add Arkady or other yeast foods to this formula (for example, see Geddes and Goulden, 1930). It is difficult to enumerate all the different variations which are now used, but obviously it is hard to describe it as a standard test much longer.

Although differences of texture count in a general way, the main results of the test are expressed in terms of loaf volume. It is, therefore, important to see what range of loaf volume there is between weak and strong flours and also what is the magnitude of experimental error, particularly when different chemists, preferably in different laboratories, perform the test on the same flour. The idea of the test is to get uniformity of results and the test must be reasonably accurate in this respect, if it is to be successful.

A great deal of work on the point has been carried out.

Swanson (1930) has given the results on four varieties of wheats which he sent for evaluation to seventeen different chemists. Of these, three used the standard baking test and ten the ordinary baking procedure. As far as this particular investigation is concerned, the chemists relying on the standard test did not, as a class, get as reliable and accurate results as the other ten.

The report of the Committee on Standardization of the Experimental Baking Test (Harrel, 1929), summarizing the result of tests on collaborative samples, states that there is considerable variation in loaf volume between laboratories and even the type of loaf varies greatly.

Treloar and Larmour (1931) investigated statistically the variability of loaf volume in experimental baking, using the standard procedure. It is significant that the final conclusions are:

"The average difference between duplicates in a large series of experimental bakes may be expected to be approximately equal to, or a little less than, the standard deviation of replicated loaf volumes. *Thus, at least three times the average difference between duplicates should be taken as the minimum difference to be regarded as significant between volumes of single loaves baked from different flours.*"

This is an important announcement as naturally we want to know, when we perform the standard tests on two or more flours, whether the difference in loaf volume is really significant.

My object is not so much to review this test as to its success in the U. S. A., but to examine its usefulness to the English chemist, who is mainly dealing with blends of wheats of varying strengths. A very wide range of strength is encountered in the United Kingdom, where, in some districts, nothing but the strongest Manitoba or hard spring wheats are used, and in others where a weaker flour with a high proportion of native English wheat is required. However, as a rule, the English cereal chemist is dealing with flours from blended wheats. To give a simple illustration (although there are normally more than two wheats in a blend), a district in which good strength is wanted might use a mixture of 75% Manitoba and 25% English, while the district where strength is not so important, might use 50% Manitoba and 50% English. To be useful to the English chemists, therefore, the standard baking test must differentiate very sharply between such wide variations and should even show significant (volume) differences with such smaller changes as the alteration of 5% to 10% English in a blend. English commercial bakers certainly notice differences of this order.

Experimental

First of all, experience in the technique was required and many tests were carried out to obtain this. When duplicate tests made on the same day gave fairly close agreement, the investigation was proceeded with. (As a rule, duplicate loaves made the same day did not vary more than 10 to 15 cc. although there might be bigger differences between the loaves from the same flour made on different days.)

A typically weak flour made from all English wheat was taken and compared with flour from a strong Manitoba. The English wheat happened to be of the Yeoman variety, which is, of course, rather stronger than normal English wheats, but, nevertheless, produces a weak flour. Then a Manitoba flour was baked, and, finally, a fifty-fifty flour mixture was taken. Averages of the three tests were taken and are shown below.

Flour	Loaf volume in cc.
Yeoman	475
Manitoba No. 2	535
50% Manitoba No. 2 and 50% Yeoman	500

All flours were untreated and, judging from volumes reported by Geddes and others, these results seem to be of the same order as those obtained in America. Nevertheless, the range in this instance did not seem very great, so that a good commercial flour was next examined. This was made from a blend of 60% Russian wheat, 20% Manitoba No. 2, and 20% Persian wheat. Such a blend was normal at the period the test was done, although usually Russian wheats are not so plentiful.

The loaf volume results of ten tests over several days varied from 520 cc. to 575 cc. with an average of 550 cc. According to this, the flour from the above blend appeared to give a larger loaf than the 100% Manitoba No. 2 and hence on the test was presumed to be stronger, which was not the case. Incidentally, this was a heat-treated flour and the addition of one milligram of potassium bromate made very little difference. Geddes (1929) has made similar observations.

Next a weak flour from a French mill was taken. At this period the mills in France were using 90% weak native wheat and 10% Manitoba wheat.

The average of seven tests gave 480 cc. in fair agreement with the 475 cc. result for the Yeoman wheat.

I then collected a series of flours from different millers from different parts of England, who made flours of varying strengths, and I have given them in descending order of strength. The blends were known to me. E.1006 was made from all Manitoba, while 0.148 had a fair proportion of weak English wheat. The average results of the standard baking tests follow:

Number of sample	Loaf volume in cc.
E.1006	555
BZ 460	565
T/t.17	545
A.569	545
M.695	540
0.148	500

From the results of the standard test I should be forced, therefore, to the conclusion that there was very little difference in strength between M.695 and E.1006, a conclusion which is certainly incorrect. In the opinion of English commercial bakers there was a very considerable difference in strength between these flours and a commercial baker used to receiving a flour similar to E.1006 would complain bitterly were he to receive one of the type of M.695. For the test to be successful in England, it should certainly be able to differentiate between any two of these flours.

From my experiments, therefore, it would appear that the test will not give the English cereal chemists, who have to differentiate between such flours, proper guidance. If the average difference between duplicates is taken at only 10 cc. then, according to Treloar and Larmour (1931), only a difference of 30 cc. would be significant in single tests. If this is so, it completely rules out the suitability of the test for English practice, seeing that such a range covers most of the flours between which one wishes to differentiate.

The suggestion of the supplementary potassium bromate test in this

case does not arise, as one wants to know from the standard baking test the value of these commercial flours as they are.

I obtained a number of flours of grindings from single wheats, such as Manitoba, Russian, Plate, Australian, Danubian, and English. Commercially, their strengths appear to be about in the order given. The average results obtained with the basic method of the standard test on these wheats were of the following order:

Wheat from which flour was milled	Loaf volume in cc.
Manitoba No. 1	560
Another Manitoba No. 1	560
Manitoba No. IV	600
Russian (No. 441)	570
Plate (Baril)	550
Plate (unknown)	485
Australian	470
Russian (No. 412 K)	525
Russian	490
Danubian	480
English	520

As in England we desire to differentiate between say a mixture of 60% Manitoba wheat and 40% English wheat, and one of 50% Manitoba wheat and 50% English wheat, it appears that the standard test, at least in my hands, is not satisfactory for such work.

When wheat evaluations are being made, it is customary in the U. S. A. to rely more upon the so-called differential test, using one milligram of potassium bromate. Following are the results obtained by this procedure. It is worthy of note that it is the high grade Manitoba flours that respond best to potassium bromate.

	Loaf volume (untreated) cc.	Loaf volume in cc. (with 1 mg. potassium bromate)
Manitoba No. 1	560	635
Another Manitoba No. 1	560	635
Manitoba No. 4	600	600
Russian No. 441	490	490
Russian No. 412	520	540
English	520	530

Discussion

I am inclined to think, at present at any rate, that, perhaps, we are tackling an insoluble problem in attempting to standardize the baking test. Different wheats and different types of flour require different baking treatments. Would the results be more reliable if a fixed water absorption were not taken? If so, who is to decide what is the correct absorption to use with any particular flour. Also, perhaps, different times of fermentation would have helped, but in a standard test this again introduces a personal element.

Besides the influence of gas production, fermentation tolerance must be considered. Manitoba flour normally has the power of producing good bread, even if the fermentation is continued beyond the time when it would normally be taken as ripe for entering the oven. This is what is usually regarded as fermentation tolerance.

Using a definite amount of yeast, for example, an English flour may produce its best loaf in three hours, and, if the fermentation is continued for four or five hours, the result is poor. A Manitoba under the same conditions may produce its best loaf in four hours, but may continue to produce good loaves when the fermentation is continued until the seventh or eighth hour.

I am aware of the work of Blish and Hughes (1932) on this subject and their view that fermentation tolerance in the U. S. A. is often confused with gas production; that some flours have been looked upon as having poor fermentation tolerance due to the fact that they failed to produce gas when the test was prolonged. This, however, does not apply so forcibly in England. Even when gas production is adequate, some flours fail to produce reasonable loaves when fermentation is continued.

The baking of loaves "thrown" at different lengths of fermentation is, in fact, the usual procedure of the Research Association of British and Irish Millers.

I have never been enamoured with the idea of trying to measure flour strength by the size of the loaf and have expressed my views on this matter on pages 171–173 of "Modern Cereal Chemistry." Such a procedure is, in my opinion, dangerous. I prefer to judge the behaviour of the fermented doughs ("Modern Cereal Chemistry," pp. 132–133). The largest tin loaves are not necessarily produced from the strongest flours. Often a strong flour to which has been added a proportion of weaker flour will produce the largest type. For example, a mixture of 80% Manitoba flour and 20% English flour will normally produce a larger tin loaf, under English conditions, than 100% Manitoba flour.

I am not, therefore, entirely surprised to find that the standard test, relying largely upon loaf volume, is not helpful in England. The volume of the "pup" loaves is, of course, the principal criterion and is, in my opinion, an unfortunate one.

Are we working in the right direction? What we really want is a test which will be reliable and which will give uniform results in the hands of different operators. The test must, of course, give information as to the behaviour of the flour in commercial baking, but, if we want standardization, need this be a baking test? From what I have seen I am very doubtful if we can standardize the baking test. If I am right, why try?

A baker desires in a flour, in addition to satisfactory diastatic activity and suitable strength, a pleasing handling of the dough. This desirable response to manipulation depends upon a number of definite, individual physical properties such as stability, elasticity, extensibility, etc., being present in a dough in a satisfactory degree. Flours are not really so much good or bad, as that they have excesses or deficiencies of various characteristics. English doughs, for example, are too distensible, but they lack stability and spring. Flours from Indian wheats are very stable but they do not have sufficient distensibility and spring. We must diagnose these characteristics in flours, which we cannot do by the baking test, which measures them all together. The baking result might be unsatisfactory because of an excess or a deficiency of any particular characteristic. A baker is naturally unable to differentiate sharply the extent and influence of each separate factor but can only record his impression of each characteristic as influenced by the others. Similarly, a baking test merely attempts to measure the final conjoint effect of all these and other factors under conditions which are themselves hard to keep constant.

In my opinion, the path of progress lies in the direction of the scientific measurement of these factors. In the first place, however, I consider that a precise definition of the various terms stability, elasticity, etc., as applied to a dough, should be agreed upon by cereal chemists of all countries. The meaning of these terms is at present very variable, since, for instance, a baker's conception of elasticity by no means conforms to the scientific definition of that characteristic.

I would particularly draw my readers' attention to the pamphlet (translation of a paper in "Mehlprobleme") "About the idea of the baking value of wheat flour," by C. W. Brabender (1932).

I do not hold any special brief for the apparatus mentioned (in fact, my experiments in this direction are unfinished), nor do I agree with everything that is stated in that pamphlet. Nevertheless, that pamphlet is logical, forcibly written and is essentially sound common sense. I commend it as a real contribution to the study of cereal science. To my mind it is hard to over-estimate the value of such a paper at a time like this.

I personally believe that one of the most promising lines of attack is in the mechanical measurements of doughs. I am not interested in any particular system. I have investigated several and they all have their good points and their drawbacks. Nevertheless, I am convinced that it is in that direction that lies salvation and not in attempting to do the impossible and standardize the baking test.

I hope I shall not be misunderstood. I shall always do a baking test in examining flours. It will be done my own way, under my own

conditions and by my own experienced test baker, who has carried out some 100,000 different baking tests on samples from all over the world. It will mean to me certain things, but it cannot be completely standardized or passed on. It is a baking test which must be adaptable and the conclusions are always supplemented by other tests. At present so many cereal chemists seem to be doing work which is misdirected. When controversial matters are being investigated, is it wise to use the uncertain and possibly misleading standard baking test? I cannot help questioning whether it is really helpful to correlate the size of "pup" loaves with protein contents. The value of high protein content under certain conditions is admitted, but there is still the quality or the colloidal condition of the protein to be considered and this may be at times the major factor. A more intensive study of this colloidal aspect is obviously wanted and I would draw attention to the significant work of de Jong and Klaar (1929, 1930, 1930a, 1931, 1932, 1932a).

In American papers there is often a reference to what has been called my theory of "optimum coagulation." To me it is more than a theory. Bearing this in mind, I can often readily see the explanation of results which appear to the authors of papers to be remarkable. A better collaboration between American and European cereal chemists would forward the cause of cereal chemistry more than anything else I know. The American chemist has specialized in certain lines and hence has collected a mass of important information on certain points. The European chemist, meeting different problems, has another view point and his research work is equally as important. In some ways, his attack is wider. He is not satisfied that a flour must be satisfactory, if the ash is low and the protein high.

If I have made this paper somewhat provocative, I have done so deliberately as I am convinced that what is wanted at the present time is plain speaking and a better understanding of other people's points of view. Do not let us flood cereal science with masses of statistics which must mean very little. Let future work be planned on really sound and logical lines.

Summary

The results of applying the A. A. C. C. standard baking test to European conditions are given and discussed. From the data obtained, these results are not encouraging and suggest that this test is not useful in evaluating the baking properties of the various types of commercial flours usually encountered. A suggestion is made that progress is most likely to be made by a more thorough study of the physical properties of doughs.

In conclusion my best thanks are due to Dr. C. W. Herd, who has carried on with the practical work whenever I have been called away, and to Dr. A. J. Amos for his help and criticism.

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SOME COMMENTS ON THE PAPER BY KENT-JONES ENTITLED "THE STANDARD BAKING TEST UNDER ENGLISH CONDITIONS"

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The paper by Dr. D. W. Kent-Jones entitled "The Standard Baking Test under English Conditions" appearing in this issue contains interesting data on the application of the A. A. C. C. basic baking test to a series of flours of varying strength. The importance in commercial bakery practice of such factors as absorption, gassing power, fermentation tolerance, and handling qualities is clearly set forth and it is pointed out that the baking test measures the final conjoint effect of all factors contributing to baking behavior under experimental conditions which are difficult, if not impossible, to standardize.

The authors are quite in accord with many of the statements made, but the paper requires some comment as the writer seems to have misunderstood the fundamental philosophy underlying the A. A. C. C. basic baking test and intimates that further attempts at standardization are more or less futile.

Kent-Jones' experiences with the basic test parallel those obtained in Canadian laboratories, recently summarized by Geddes and Larmour.[4] The spreads in loaf volume yielded by different flours were not great and in many instances the results of the basic test, considered alone, would have led to preposterous deductions in regard to relative strength. Moreover, the Canadian laboratories collaborating in the work of the Associate Committee on Grain Research, National Research Council of Canada, have not followed the A. A. C. C. procedure precisely as outlined. In the first place it was found impossible to use a fixed amount of water for mixing and consequently they have always added water to suit the requirements of the dough. Then it was found that the low-sided pans gave larger loaf volumes and greater differentiation between flours and these were adopted. Furthermore, Canadian cereal chemists have found it desirable to employ several formulas that are different

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from any of those suggested by the Baking Test Committee. In short, they are quite unorthodox as far as the absolute standard baking test originally agreed upon is concerned.

Despite these departures from orthodoxy, they consider themselves supporters of the fundamental principle behind the attempt to standardize test baking because whatever modifications are applied, they are superimposed on a basic procedure which is familiar to all those using the standard method and for that reason the results can be interpreted, to some extent at least, in terms of common experience. This common experience rests on the use of the same quantities of flour, salt, and yeast, and the same conditions of fermentation and baking with respect to times and temperatures. By fixing these variables it is possible to describe briefly any modifications of formula or procedure which may be introduced for specific reasons, and to describe them in such a way that the reader who is familiar with the basic procedure can get a very clear idea of the test methods used. It seems to the writers that upon such a standard skeletal procedure, one can superimpose a sufficient number of variations to secure all the information required about a particular flour and at the same time work from a common basis which will permit others to interpret the results, to some extent at least, in the light of their own experience.

It would appear from Kent-Jones' paper that the basic test is considered *the* standard baking test, that the use of supplementary tests is to be regarded as a step towards de-standardization and an indication of the futility of making further attempts to standardize baking tests. This viewpoint, the authors believe, is due to a misinterpretation of the function of the basic procedure as set forth by Blish on many occasions. As Chairman of the Baking Test Committee in 1928 he emphasized that the basic test was merely a foundation and reference point and recommended several supplementary tests. Hence, early in the work of the Baking Test Committee, it was clearly recognized that no single formula would reveal all the baking characteristics of a flour. The authors of these comments do not regard the introduction of supplementary tests as a step towards de-standardization but rather as an admission that several formulas are necessary.

In dealing with the question of standardization there are two distinct considerations: first, the development of standard baking formulas designed to reveal specific information; and secondly, the development of a standard technique in regard to operating details.

Unfortunately, there is not general agreement among cereal chemists as to the type of information desired from a baking test. Some desire a test which will reflect the joint effects of gluten strength and natural

diastatic activity; others desire a test which will furnish a measure of strength independent of gassing power; still others wish to measure tolerance to mixing or fermentation. Again, some cereal chemists, as in Europe, are more interested in the behavior of a flour in regard to dough characteristics, such as toughness, elasticity, freedom from stickiness, to determine the facility with which large masses of dough can be handled in the commercial bakery. No single test baking method can meet these varied demands and the supplementary procedures specified by the A. A. C. C., in conjunction with the basic test, as a reference point to assist in their interpretation, are designed to provide for these different requirements.

Some information regarding handling quality is obtainable by the manual operations necessary during the baking process. This, however, is not regarded by American cereal chemists as the major object of experimental baking tests; in general, the flours with which they have to deal are satisfactory in this regard.

The efforts of the Baking Test Committee have perhaps been directed more towards standardizing the operating details of the test than to a consideration of the development of baking formulas to reveal specific information. The extensive work conducted on the mechanics of the test has elucidated the importance of such variables as absorption, mixing, punching, and molding, and size and shape of pan. The results clearly emphasize the necessity for standardizing the baking technique and much has been accomplished towards minimizing the personal factor. It is logical that the operative details should be standardized before intensive studies with a view to developing formulas and interpreting the results in terms of gluten quality or gassing power may be undertaken.

The lack of agreement among cereal chemists as to the purpose of baking appears to be responsible for much of the criticism that has appeared in several recent papers. In the abstract sense, it can be stated that the purpose of test baking is to secure information which may be used to predict the behavior of a flour under any and all conditions to which it may be submitted in commercial or home bakery practice, to determine wherein the flour is deficient, and to provide the basis for specifying the particular conditions under which it will give optimum results. To secure such complete information involves the use of a number of baking formulas designed to measure the joint effects of gassing power and gas retention, the gas retention factor only, the tolerance to mixing, to prolonged fermentation, and to oxidizing agents. When the baking methods under which a flour is to be used in practice are known, such a complete study of its characteristics need not be undertaken.

The problem of the mill chemist who is generally required to evaluate flour with reference to some particular commercial baking practice, is much less complicated than that of the cereal chemist who is called upon to investigate the quality of new wheat varieties submitted by plant breeders. In the latter instance, a standard skeletal procedure is necessary as a starting point. The basic test is an effort to meet this requirement and its limitations are now generally recognized. Much intensive work still remains to be done in developing supplementary methods the results of which can be definitely interpreted in terms of the adaptability of the flour to any particular set of commercial baking conditions.

It has been suggested that the various factors contributing to baking quality might be more satisfactorily evaluated by means other than baking, such as mechanical measurements of doughs. For years the efforts of cereal chemists have been directed towards this end. Physical, physico-chemical, and chemical factors have been studied, but as yet it has been impossible to replace the baking test by quantitative measurements of these factors. The Brabender farinograph is the most recent and probably the most highly perfected apparatus for making mechanical measurements on dough. Such measurements must, however, be interpreted in terms of baking behavior and hence, in the last analysis, the baking test is the standard of reference which must be employed. The very fact that baking behavior is a conglomerate of a number of factors would seem to render it very difficult, if not impossible, to predict with precision the baking characteristics of a flour from a series of quantitative values which may vary independently. While the baking test has many drawbacks, it must remain until our knowledge of cereal chemistry greatly increases; for maximum utility, the procedure and interpretation should be standardized. The progress already made, while less rapid than hoped for, amply justifies the active prosecution of further studies.

THE LABORATORY BAKING TEST—A SCIENCE OR A FINE ART?

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In this issue of Cereal Chemistry appears a paper by Kent-Jones in which the standard A. A. C. C. laboratory baking test is made the object of destructive criticism. Although the discussion is represented as applying especially to European conditions, it is concluded that cereal technologists *generally* should abandon any systematic attempt to apply to the laboratory baking test the same sound scientific principles that are nowadays accepted without question in the testing of other important materials.

One is likely to infer from Kent-Jones' discussion that flour quality is a mysterious "something" that is quite beyond the range of comprehension and methods of ordinary laboratory technicians and that only by the artistic touch of a talented, expert baker can it be usefully ascertained and evaluated. Especially is it considered necessary to revert to the old "artistic touch" method when handling flours milled from *blends* of wheats of different types and from different localities.

There is a considerable group of cereal technologists that is inclined to regard ideas of this character as distinctly reactionary. When these ideas are publicly expressed by a recognized authority in the field of cereal chemistry they are likely to impede scientific progress along lines where progress and enlightenment are badly needed.

The need for a common yardstick for the measurement and classification of those flour properties that constitute "quality" is perhaps felt less strongly by the average mill chemist than by the "agronomic" technologist, who is forced to deal with the small samples that are submitted for evaluation by the agronomist and the plant breeder. Here it is necessary to consider "quality" in an abstract sense, and not with reference to any specific type of commercial baking procedure.

The commercial interests have on numerous occasions clearly indicated that they recognize the fundamental importance of developing the best possible wheat varieties. However, unless the industrialist and the agronomist have a common basis for discussing "quality," they can never hope to arrive at mutual agreement and understanding. This has

been abundantly demonstrated whenever the agronomist has submitted the same samples to each of several different laboratories for quality rating.

At this point it is appropriate to re-emphasize the fact that the responsibility of cereal technology, as a field of science, goes much farther than the mere testing of flour as to its ability to meet the specific requirements of some individual baker or group of bakers.

As was stated by Blish and Werner,[1] the cereal chemist must "acquire the ability to ascertain, identify and classify the properties of the materials *as he finds them*. . . . Any satisfactory scheme for the useful identification and classification of properties of matter depends, first of all, upon the establishment of reliable and scientific methods of analysis or ascertainment. When the material under consideration is highly complex in composition, and when, in addition to this, the only analytical method available is such that several environmental factors are involved, it becomes highly essential that a *standard* and fixed and, if needs be, arbitrary procedure be established and uniformly employed, if classification, in terms of common understanding, is ever to be realized. There is no other known basis for a standard system of classifying the properties of matter."

Platt [2] recently said: "In making a determination of baking quality, it is highly desirable and indeed necessary to start with one standard baking test, giving one result."

Surely it must be obvious to all who wish to promote the dignity of cereal chemistry as a special department of biochemistry, that the adoption and development of the standard A. A. C. C. laboratory baking test means far more than merely the tentative acceptance of an analytical procedure. It means the endorsement of a *philosophy*,—the same type of philosophy that has been responsible for the present establishment and compilation of the official "Methods of Analysis" of the Association of Official Agricultural Chemists. All agricultural analytical methods considered worthy of being designated as "official" are *fixed* and *arbitrary* methods, established as such only after years of careful work and collaborative study.

No one will deny that the standard A. A. C. C. method, itself, is far from having reached anything approaching perfection. It simply represents a determined and organized step in the right direction. It duly recognizes the purpose and the limitations of its so-called Basic Procedure, which is to be regarded merely as a point of reference and a basis for any supplementary tests that may be considered appropriate.

The experiments upon which Kent-Jones appears to have based his

[1] Blish, M. J., and Werner, E. E. The cereal chemists' responsibility. Northwestern Miller, 1929, **157**: 118.
[2] Platt, Washington. 1933. Higher versus lower mathematics in interpreting baking quality. Cereal Chem. **10**: 213–222.

conclusions are decidedly limited in their scope, and they involve false assumptions. He assumes, for example, that the American chemists base their criteria of "strength" exclusively upon differences in *loaf volume,* using the basic procedure alone, although he, himself, has "never been enamoured with the idea of trying to measure flour strength by the size of the loaf."

This is decidedly an unwarranted assumption. If there is any one thing that experience with the basic procedure has impressed upon its users it is the fact that flour strength *cannot* be judged by loaf volume alone. Indeed, conditions are frequently encountered where loaf volume is *negatively* correlated with "strength," when dealing with the basic procedure alone. Certain aspects of this situation have been repeatedly pointed out by cereal technologists both in the United States and in Canada.

In discussing the "path of progress" in flour quality testing, Kent-Jones would emphasize those properties that, in the aggregate, constitute what is usually designated, rather loosely, as "gluten quality." His personal belief is "that one of the most promising lines of attack is in the mechanical measurements of doughs." Surely there is nothing new or controversial about this idea, which has been shared by others for many years. All sorts of mechanical devices have been proposed and employed in studies of this character, the Brabender Farinograph being one of the most recent and possibly the most highly perfected type of mechanical contrivance for its intended purpose.

One might infer from Kent-Jones' discussion that the standard A. A. C. C. test fails to provide for "mechanical measurements of doughs," but the actual fact is quite to the contrary. Supplementary Method D is intended exclusively for that specific purpose. True enough, there are as yet no detailed specifications for the performance of Supplementary Method D. These may come later. As before stated, but as many still fail to comprehend, the standard test is not proclaimed to be finished in all of its aspects, but is merely an "organized step in the right direction," and until the various supplementary methods are specified and generally agreed upon, all that can be done is to establish the common *reference point,* namely, the Basic Procedure.

Any supplementary method may be used for any purpose whatsoever, without violating the purpose and intent of the standard A. A. C. C. test, providing only that Basic Procedure is used as the point of reference. Of course it is essential to indicate the nature of the supplementary method used, and to report loaf characteristics produced both by the Basic and supplementary methods if any intelligent basis for a common interpretation of results is to be provided.

This situation does not seem to have been appreciated by Kent-Jones

in carrying out the experiments upon which his present criticism is based. If he was primarily interested in "gluten quality" (based upon physical and mechanical dough properties) as affected by different blends, why then did he not use, in some form or another Supplementary Method D, which is specifically indicated for such purposes?

He failed to use the very tool that the standard A. A. C. C. test provides for his intended purpose, and then criticizes the test as being inadequate. This situation tends to destroy the premises upon which his attack upon the standard A. A. C. C. tests are largely based.

Let it once more be reiterated that the standard test is still in its elementary stages. Its adoption in present form by the A. A. C. C. is merely the endorsement of a *philosophy* and a systematic attempt to establish a common reference point, which is the Basic Procedure. It is frankly admitted that even the Basic Procedure may be subjected to later modifications and improvements. It is also duly recognized that many who now pretend to use the test do not adhere strictly to *all* of the established specifications for the Basic Procedure.

In spite of all this, many reputable cereal technologists have noted a distinct improvement over the chaotic situation which formerly prevailed when they attempted to find a common basis upon which to discuss flour and bread characteristics. This was especially prominent in private discussions among certain groups of cereal chemists at the recent Chicago convention. In these discussions several chemists commented individually and specifically upon the substantial advancement in this direction. Among users of the "pup" tests there now exists a basis for mutual understanding in the communication of ideas on flour and baking characteristics that is highly encouraging to those who recall former situations where each man spoke a different language, and where the artistic skill of the individual technician very largely determined whether the flour should be considered of good or bad "quality."

This improved condition exists in spite of the fact that there are variations in manipulation among many who habitually use the A. A. C. C. test. The A. A. C. C. baking test standardization project has therefore been well worth while even though there is not a 100% uniformity in manipulation.

Even a partial standardization has been found to be far better than none at all. There is every justification for encouraging further effort along lines already started. Mechanical contrivances may usefully supplement the baking test, but it is not likely that they will entirely replace it within the present generation. The standard A. A. C. C. baking test represents an organized attempt to remove flour quality testing from the realm of the fine arts, and place it in the domain of scientific endeavor. Efforts to undo what has already been accomplished should not be tolerated.

CEREAL FOODS, THEIR ADVERTISING AND THE COMMITTEE ON FOODS OF THE AMERICAN MEDICAL ASSOCIATION

RAYMOND HERTWIG

Secretary, Committee on Foods, American Medical Association, Chicago, Illinois

(Read at the Convention, June, 1933)

The Committee on Foods has been delegated by the American Medical Association to pass on foods and food advertising; to exert the influence of that Association in support of foods of desired and required nutritional values and of proper and truthful food advertising in the interest of public welfare and health. The work of the Committee is constructively educational. Nationally and locally distributed foods are considered alike irrespective of manufacturer or merchant. Sponsors of foods accepted by the Committee assume the responsibility of merchandizing the products wholly in accord with the Committee's Rules and Regulations and General Committee Decisions which are published in booklet form and available to all. Food brands not fulfilling requirements as foods or promoted by improper untruthful advertising are subject to rejection. Actions of the Committee, favorable or unfavorable, are published; reasons for adverse action are stated.

The Committee personnel comprises:

Morris Fishbein, M.D., Editor of The Journal of the American Medical Association, Chairman.
Lafayette B. Mendel, Ph.D., Sc.D., Professor of Physiological Chemistry, Yale University, Vice-chairman.
E. M. Bailey, Ph.D., Chemist in Charge, Analytical Laboratory, Connecticut Agricultural Experiment Station.
Julius H. Hess, M.D., Professor of Pediatrics, University of Illinois School of Medicine.
Philip C. Jeans, M.D., Professor of Pediatrics, University of Iowa School of Medicine.
Edwin O. Jordan, Ph.D., Sc.D., Professor of Bacteriology, University of Chicago.
James S. McLester, M.D., Professor of Medicine, University of Alabama.
Grover F. Powers, M.D., Professor of Pediatrics, Yale University.
Mary Swartz Rose, Ph.D., Professor of Nutrition, Teachers College, Columbia University.
Russell M. Wilder, Ph.D., M.D., Professor of Medicine, Mayo Foundation, Mayo Clinic.
Raymond Hertwig, B.S., Secretary of the Committee.

The work of the Committee as it affects cereal foods and their promotional advertising will only be considered. Any cereal food is eligible for acceptance under the conditions:

(1) That it be submitted as required by the Rules and Regulations,
(2) That it fulfills the desired and required nutritional or other values for the respective type of food article,
(3) That it be wholesome,
(4) That it is not in violation by adulteration or misbranding of the Federal Food and Drugs Act, and
(5) That the label and advertising accord with the Committee's Rules and Regulations and General Committee Decisions.

A submission for a cereal food includes:

(1) Samples, labels, and all pieces of advertising,
(2) Manufacturing formula for mixtures or compounds,
(3) Analysis defining the chemical composition or substantiating any unusual chemical claim,
(4) Definition or specifications for the raw materials or ingredients used in preparation of the article,
(5) Description of manufacture and packaging, and
(6) Confirmation of vitamin content in instances of specific vitamin claims.

A complete submission permits the Committee to understand and appraise a product thoroughly and to pass on the advertising scientifically.

Accepted cereal foods include one or more examples of almost every type of simple or compound cereal on the market. In round numbers 650 cereal products have been acted on; 450 have been accepted, 35 rejected, 150 are pending, and 15 acceptances have been withdrawn. The acceptance of practically every product involves some label and advertising change; in many instances the labels and advertising are drastically or completely revised. The influence of these changes throughout the cereal food field is unquestionably far-reaching. It is the purpose of the Committee to increase the influence and power of good advertising and decrease that of the bad; to increase public acceptance of foods of desired and required nutritional values and decrease public acceptance of foods of undesired and inferior nutritional values. Society is dynamic; its standards represent an equilibrium between destructive and constructive forces. The law of the land tends to maintain society in status quo; public welfare activities such as those of the Committee tend to raise society to new and higher levels of human good and health. It is not strange, therefore, to find an organization such as the American Medical Association in this social and economic struggle for improving the health of the nation through foods and food advertising.

Inappropriate Types of Advertising

Examples of some of the common inappropriate types of advertising and claims not permitted for accepted cereal products either because incorrect, not in accord with best trade practices, or not supported by satisfactory experimental evidence, and some requirements for cereal foods will be mentioned.

Flour

"Makes more loaves"; "makes cakes and bread that keep fresh longer"; "contains all the nourishment of wheat"; designations such as "patent," "highest patent" or "short patent" for "straight" or mixtures of "straight" and "clear" flours; grade designations without general established significance; specific superlative claims of quality; omission of bleaching declaration from labels; derogatory statements directed at bleached flours; and unfair competitive claims, have been subject to adverse action by the Committee. The only recognized designation for flour grades are "straight," "standard patent," "patent," "short patent," and "clear."

Whole Wheat Grain and Cereal Products

Whole wheat is the stronghold of food faddists; "health food" advertising has been and is especially rampant for whole wheat foods. Why whole wheat has been singled out for sanctification by food cultists is apparently more a problem for psychoanalysis than for scientific nutrition. Specific health food claims and food faddism advertising are disallowed for accepted foods. Incorrect and vague vitamin and mineral claims are frequent for whole wheat products. Such claims should be specific and name the vitamins and minerals referred to. Whole wheat cereals do not build strong bones and teeth nor cure constipation. Foods providing considerable bulk in the intestines tend to prevent constipation due to insufficient bulk. Elements necessary for proper anabolism of bones and teeth are not provided by whole wheat or not in propitious quantities. Chewing whole wheat crackers and "hardtack" does not produce good teeth and healthy gums. Good teeth and healthy gums depend on proper nutrition and not on chewing. Whole wheat cereals are not reducing foods nor are they especially adapted to carbohydrate restricted diets. The terms "whole wheat" and "graham" are to be used as food names or parts of food names only when the sole cereal and farinaceous ingredient is whole wheat.

Breads

"Skim-milk" is frequently called "milk" in listing bread ingredients on labels and in advertising. The claim "made with milk" is proper only for a milk bread and not for bread not complying with the U. S. Department of Agriculture definition for milk bread. Statements of ingredients of bread should be complete and the ingredients should be listed in the order of decreasing proportions in the formula. This is a basic requirement for all compound foods. Descriptive names

of breads should be compatible with the composition. Breads containing unexpected ingredients should bear label declarations to that effect. The public is entitled to know the ingredients of the foods it consumes. The addition of drugs, as senna and phenolphthalein, to breads is not permissible. Drugs have no place in foods. Breads prepared from unusual ingredients with relatively low carbohydrate content merit claims for carbohydrate restricted diets but not for reduction diets. There are no diabetic breads; the term diabetes or other disease names should not be used in the advertising for any breads. All foods of high caloric value will fatten if consumed in greater quantity than required for meeting maintenance energy requirements. There are no "health breads"; white bread is as much a health bread as is bran bread. A "butter bread" contains butter as the only shortening. A "butter cream bread" has not been defined but would probably contain more milk fat than practical to incorporate in bread. A previously accepted "butter cream bread" will be rejected unless the name is made appropriate in the future.

The Committee has accepted the educational advertising of the American Institute of Baking. That Institute deserves special mention for the support it is giving the Committee for promoting sound advertising. It is an example of the excellent cooperation being offered by several trade associations. The milling and baking industries as a whole are to be commended for their earnest effort for establishing straightforward honest advertising. That the Committee has given them support is shown by the large number of accepted cereal foods.

General Cereals

Farina is the first cereal food for babies but not the first solid food for babies. Vegetables now precede cereal in many infant diets. Vitamin claims for breakfast cereals should be correct and specific. Vague vitamin claims are misinformative as are vague mineral claims. The term "sterilized" should not be used for cereals merely heat treated to destroy insect infestation. No marketed cereals are sterile. Constipation claims for bran cereals should be restricted to some such claim as "an aid for counteracting constipation due to insufficient bulk in the diet."

It is pertinent at this point to quote two General Committee Decisions, on "the ideal food label" and "good food advertising," recently adopted for promulgation to food manufacturers and the public. These decisions positively and constructively present the Committee's views on food labels and advertising:

The Ideal Label for Foods

(General Committee Decision)

"The container label of foods should conspicuously present such information as will properly inform the public of the true nature and quantity of the food within the package. The public deserves to know the identification of the foods it purchases. There are no sound arguments justifying secrecy on the composition of foods. Label identification of foods is a most cogent influence for prevention of incorrect, deceptive, or fraudulent advertising apart from the package container. A properly informative label lays the basis for good sound advertising, the only kind of advertising the public or the food industry can permit in their own interest.

"Accepted foods, among other things, are intended to serve as examples of foods properly labeled in the interest of the public and of the food industry as a whole. As such examples they militate for the adoption of properly labeled foods throughout the food field and of good equitable advertising and competitive practices.

"An illustrative diagram of an ideal food label is presented for the guidance of food manufacturers and represents the type of label the public expects in its own welfare":

IDEAL LABEL FOR FOODS

(Skeleton outline for main panel faces)

COMMON NAME OF FOOD
*(Statement of added minor ingredients)

Example:

RICE FLAKES
*(Flavored with sugar, malt and salt)

—OR—

FANCIFUL TRADE NAME
*(Descriptive statement identifying ingredients)

Example:

BLANCO
*(Sugar, dried fruit, eggs, and milk)

ADDITIONAL INFORMATION OF A SPECIAL CHARACTER

NET CONTENTS

NAME OF MANUFACTURER, PACKER, OR DISTRIBUTOR

* NOTE: Ingredients arranged in order of decreasing proportions.

According to the Committee's decision, the ideal food label presents on the main panel faces the common name of the food article accompanied by a statement of any added minor ingredients; for example, "Rice Flakes—flavored with sugar, malt and salt." A fanciful tradename for a food is accompanied by a descriptive statement identifying the ingredients; for example, "Blanco—contains sugar, dried fruit, eggs, and milk." The ingredients are listed in the order of their decreasing proportions in the product to prevent giving undue prominence to ingredients present in small amounts. The net contents and the name of the manufacturer or packer are declared on the main panel faces.

Good Food Advertising

(General Committee Decision)

"Food advertising must be considered from the points of view of both the public and the food merchandiser. Sound advertising effectively serves the interests of both. The continued welfare of the food industry rests largely on the dedication of its advertising activities to the good of the public. It is essential therefore to define proper food advertising.

"Proper food advertising should use the common name of the food concerned, or in the case of a fanciful tradename should identify the ingredients in the order of their decreasing proportions in the product. Such practice prevents deception. Any statement of the physical, chemical, nutritional, or physiologic properties and values of the food should be truthful and expressed in simple common terms. Proper advertising is free from false implications. It does not create incorrect or improper inferences or comparisons between foods. It attempts to promote sales solely on the merits of the food article itself.

"Good food advertising harmonizes with established authoritative knowledge popularly expressed. Meritorious foods require no exaggerated, false, misleading claims. The inferior food with alleged fictitious values requires gross superlatives and exaggerations, and flamboyant, vague and mysterious claims. Good advertising discusses nutritional values but avoids specific health claims; it recognizes that health depends on the diet as a whole and on many factors other than foods and not on any one food brand nor any one type of food."

The Committee is enlisting the cooperation of the food industry for developing a system of self-control by which the industry from within itself will be governed only by established knowledge, the welfare of the industry and the consciousness and recognition of public welfare. With such a system and on this basis only can the food industry serve its own

best interests and those of the public. Progress is being made. The industry is largely made up of that type of men who will not fail when they once have defined their problem and purpose, envisaged their goal, and have faith in their self-appointed leadership. The service of the nation and of mankind has become an integral part of economic success. Material success without service to humanity is mockery. The glow of life is in service.

The American Association of Cereal Chemists through its ideals and its scientific endeavors is impregnating the entire cereal industry with its own spirit. The cereal industry looks to your association not only for technical guidance but for leadership in ways and means of efficiently serving itself and the nation in the manufacturing and merchandizing of cereals.

DESTROYING MOLD SPORES ON BREAD BY ULTRA-VIOLET RADIATION

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(Read at the Convention, June, 1933)

According to the United States census figures for 1929, there were 20,785 establishments in the United States engaged in the baking of bread and other bakery products. The aggregate sales of the products manufactured totalled $1,526,110,811. The cost of materials, containers for products, fuel, and purchased electrical energy was $737,099,500. The difference, $789,011,311 represents the value added by manufacture. On the basis of this value added by manufacture, the industry ranked seventh. The industry suffers an annual loss of several millions of dollars due to the spoilage of goods caused by several types of common molds. Different methods of reducing these losses have been devised but no one of these has reached general application, aside from taking extreme precautions to keep the bakeshop clean and sanitary.

In their studies dealing with molds found on bread Herter and Fornet (1919) reported the occurrence of the following species of *Aspergillus,* viz., *nidulans, glaucus, niger, candidus,* and *fumigatus.* Among the *Penicillia* they found the species *glaucum, crustaceum,* and *oliviaceum;* and, of the *Mucor* type, the species *pusilus, stolonifer,* and *mucedo;* and lastly, the pink mold, *Monilia sitophila.*

Streider and McClellan (1922) reported an extensive study dealing

with molds in bakeries. The types of molds which they found consistently on moldy bread were *Aspergillus, Penicillium,* and *Rhizopus.* The appearance of *Mucor* was more or less sporadic during the winter season, but was frequent during the summer. Species of *Rhizopus* and *Aspergillus* were generally present.

Within the past five years several workers have investigated the fungicidal properties of ultra-violet light. The most thorough study was made by Fulton and Coblentz (1929). They exposed twenty-seven miscellaneous species of fungi for one minute at a distance of six inches. The molds were propagated to maturity in Petri dishes and were subjected to irradiation in these dishes. The source of ultra-violet was a 110-volt mercury in quartz arc lamp consuming approximately 320 watts (80 volts + 4 amperes) in the burner. The ultra-violet energy emitted by this lamp at a distance of six inches for one minute completely killed 16 species out of the 27 exposed. An exposure of five seconds at six inches killed 907 out of 1000 spores of *Penicillium digitatus* and a 45 seconds' exposure killed 998 out of 1000. Spores of *Penicillium digitatus* and *Rhizopus nigricans* were completely killed after one minute exposure. The resistant groups which required much longer exposures to kill with the intensity obtainable from this lamp had dark spore walls. These are difficult for the rays to penetrate. The killing effect was cumulative. Several short intermittent exposures were practically equivalent to one continuous exposure.

Welch (1930) reported studies on the effects of ultra-violet light on molds, toxins, and filtrates. He used a "C" carbon lamp. It is assumed that this was the ordinary C_4 type, operating at 60 amperes and 50 volts without screens or reflectors. Eleven strains of *Aspergillus,* 14 of *Penicillium,* and 11 of *Mucor* were subjected to the radiation from this lamp for varying lengths of time at a distance of 10 inches. He found that *Aspergillus niger* required the longest period to kill. Also, that certain species of molds develop a resistant group of spores. Exposure for one minute destroyed more than 90% of the molds in each culture, while the rest persisted considerably longer. Irradiation for 2 minutes destroyed all *Mucor* species and also all species of *Aspergillus* and *Penicillium* except the resistant spores of certain species among these latter two types.

In a more recent paper Owen (1932) makes claims for the successful application of ultra-violet radiation as a practical means of materially reducing the ravages from mold which occur in connection with the marketing of sliced bread. He did not secure complete sterilization of sliced bread even after three minutes' exposure to the special carbon lamp which he used, but this period of irradiation reduced infection to practically one-fourth of that noted on his controls. He also reported

that irradiation for thirty seconds injured the spores sufficiently to cause them to require about twice the length of time to germinate. The distance from the lamp at which his exposures were made was not given.

Coblentz and Fulton (1924) made a radiometric investigation of the germicidal action of ultra-violet. Their source was a 110-volt mercury in quartz arc lamp which was operated at 80 volts and 4 amperes. Exposures were made at a distance of six inches. In order to kill a bacterium in one second, the authors calculated that the radiant flux of the germicidal radiation must exceed a certain threshold value, which is of the order of 25 microwatts per square millimeter, or its equivalent, 250 ergs per square millimeter per second. At a distance of 8 inches instead of 6 inches the erg output would be reduced from 250 to 140. Using the average surface area of a bacterium subject to exposure, they further calculated the quantity of ultra-violet energy required to kill one bacterium in one second. This was found to be equivalent to 4.5×10^{-12} gram calories or to 18.8×10^{-5} ergs, which represents the energy impact of ultra-violet rays from 1700 to 2800 Å [1] required to destroy one bacterium in one second at a distance of six inches from the mercury vapor burner employed by Coblentz and Fulton.

Discussion

The destruction of fungus spores by short wave irradiation is far more difficult to accomplish than is the destruction of bacteria. The rays which kill are those absorbed by the body acted upon. The action appears to be a direct one, due to the absorption of the lethal rays by the protein molecule. There is some evidence for believing that certain amino acids in the molecule are specifically attacked and disrupted. The quick killing wave lengths appear to range between 2950 and 2400 Å.

In the News Supplement to Science, March 31, 1933, attention is called to a motion picture demonstration showing how certain wave lengths of ultra-violet kill cells. Living cells from the spleen were subjected to wave lengths ranging from 4350 to 2253 Å. The wave lengths that were fatal to the cells killed in from 15 to 20 seconds. The living protoplasm became greatly agitated, bubbles appeared on the cell membrane and, as a rule, the cells finally burst. It was stated that the killing effect of the shorter ultra-violet light was not equalled by fifteen hours of exposure to strong radiation from radium, nor was the lethal effect of ultra-violet equalled by 12 to 24 hours of exposure to high voltage X-rays.

In general, the relative amount of ultra-violet energy emitted by

[1] Ångström units.

wave lengths below 2900 Å is rather small when compared to the total amount yielded by a given source. In the case of the mercury arc spectrum, the emission below 2900 Å is less than 6% of the total. A comparison of the fungicidal emission from the mercury vapor burner to that yielded by certain carbons reveal some interesting figures, evaluated as ergs per second per square millimeter.

For the "C" carbons at an 8-inch distance	Irradiation shorter than 2700 Å.	Irradiation shorter than 2900 Å.
Operating at 60 amps. and 50 volts	1000	1250
Operating at 90 amps. and 50 volts	1750	2250

For the "U" carbons at an 8-inch distance	Irradiation shorter than 2700 Å.	Irradiation shorter than 2900 A.
Operating at 60 amps. and 50 volts	750	1250
Operating at 80 amps. and 50 volts	875	1500

In the final experimental tests with different carbons the lamp was operated on direct current at 75 amperes and 60 volts. This would give an energy output in ultra-violet below 2900 Å, approximately half way between the two values shown above for the "C" carbon, or 1750 ergs per second per square millimeter. Over a period of 45 seconds, the time required to exterminate mold spores with the "C" carbon operating at 75 amperes and 60 volts, at a distance of 8 inches, the energy emission per square millimeter would be 45×1750 ergs, or 78,750 ergs. This is 315 times the energy factor required to exterminate bacteria as calculated by Coblentz and Fulton.

Experimental

An experimental study was made of the fungicidal potency of the radiation from three models of the mercury vapor lamp and from the open C_4 type of the carbon lamp without reflectors or screens. The mercury vapor lamps employed were:

(a) A standard model operated at 90 volts and 5 amperes,
(b) A Super model, equipped with a highly polished special reflector, and operated at 95 volts and 5 amperes, and
(c) A Uviarc model with General Electric No. 10 burner, operated at 220 volts and 5 amperes.

Freshly baked bread was heavily infected with the mature spores of the more common species of *Aspergillus, Penicillium, Rhizopus,* and *Mucor,* and subjected for varying lengths of time at given distances to the ultra-violet radiation from the different sources employed. The in-

fected loaves were then wrapped in sterile waxed paper and incubated at 93° to 95° F.

The ultra-violet intensity of the first two mercury vapor lamps was not sufficient to inactivate the spores in a short enough time, at a distance of six inches, to show any commercial promise. Using a Gordon ultra-violet photometer the Super model gave a total energy reading of 226 ergs per square millimeter per second at a distance of six inches, equivalent to about 14 ergs per square millimeter per second for wave lengths below 2900 Å. The Gordon ultra-violet photometer is an instrument which is only approximately accurate.

The total energy output from the Uviarc lamp with No. 10 burner is approximately 2600 ergs per square millimeter per second at 20 inches, and 7000 ergs at 7 inches. On the basis that only about 6% of the total ultra-violet energy from the mercury arc is emitted by wave lengths below 2900 Å, the fungicidal energy impact from this lamp at 20 inches and at 12 inches would be respectively 156 and 420 ergs per square millimeter per second. These evaluations are based on radiometric measurements. Thus the General Electric No. 10 burner operating at 220 volts emitted approximately 120 times more energy below 2900 Å at a distance of six inches than did the Super model. The No. 10 burner proved to be quite destructive to mature spores at a distance of six inches. Exposures for 1 minute at this distance generally caused complete destruction of all spores.

For commercial use the mercury vapor lamp does not appear to offer the advantages to be obtained by use of the carbon lamp. The carbon lamp is capable of emitting a much higher intensity of the shorter fungicidal wave lengths. Carbons may be selected especially for their high intensity in the lethal zone. The fungicidal action of the "C," "U," "K," and "Magnesium" carbons was investigated.

The "C" carbon contains a mixture of materials in the core, including iron, nickel, and aluminum. Radiation from this carbon is characterized by a series of groups of very intense lines through the ultra-violet spectrum. "K" carbons are cored with cobalt as the principal flame material, and these give a slightly higher intensity of radiation near 2400 Å, also a peak near 3600 Å. The "magnesium" carbon yields a very high peak at 2800 Å, and the "U" carbons give a high intensity of ultra-violet below 2900 Å.

Results obtained with the "C," "U," "K," and "magnesium" carbons showed decreasing fungicidal potency in the order named. Lethal rays from the "C" carbons destroyed heavy spore infection on the surface of the loaves in 45 seconds at a distance of 8 inches with the lamp operating on direct current at 75 amperes and 60 volts. Under the same conditions of operation the "U" carbons ranked second in

fungicidal power. The ultra-violet from the "U" carbons required 60 seconds to accomplish what the "C" carbons did in 45 seconds. Positive carbons were 22 millimeters in diameter and negative carbons 13 millimeters.

Another interesting phase of this investigation was the irradiation of infected loaves through transparent sheeting such as Cellophane and Sylphwrap. In air, wave lengths around 2400 Å were cut off by the viscose sheeting having a thickness of approximately .025 inch. This fact, however, did not show any practical effect on the fungicidal potency of the radiation. Under the same conditions the mold spores were destroyed by the radiation which passed through the transparent sheeting just as readily as if they were on the uncovered surface of the loaf.

This process of sterilization is now being successfully employed on a limited commercial scale to prevent the occurrence of mold on certain special products such as Proteo bread, a bread scientifically formulated for diabetics, and for those on restricted diets.

Proteo bread is baked in a round, crimped crust pan. Since the loaf is round it is necessary to rotate it during exposure to the light in order to secure uniform irradiation of the entire longitudinal surface. This is accomplished by equipment especially constructed for the purpose. In practice, the revolving loaf is irradiated for two minutes. In this time it makes two complete circuits around the carbon arc and revolves on its own axis approximately ten times. In spite of the fact that the corrugated surface of the loaf offers more or less protection to the spores, a two minute period of irradiation has proved commercially practical.

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SOME EFFECTS OF HEAT EXPOSURE ON WHEAT STARCHES [1]

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(Read at the Convention, June, 1933)

Starch has an important rôle in bread and other cereal foods. In bread doughs, starch is important not only as a potential source of sugar, but also has an important function in forming the cellular structure of the baked product. The properties of the starch are obviously important in macaroni and similar products.

Kent-Jones (1927) noted an improvement in baking quality of flours exposed to moderate heat treatment. From chemical studies on heat treated flours, he concludes that (1) heat treatment decreases peptizability of flour proteins; (2) heat treatment increases imbibitional properties of flour proteins; and (3) that with moderate heat treatment no experiments have revealed any perceptible alteration of the starch.

Geddes (1929, 1930, 1930a) has made extensive studies on chemical and other changes induced by heat treatment of flour. He agrees with Kent-Jones that heat treatment decreases the peptizability of flour proteins, but considers the changes induced by heat as due principally to oxidation of lipoid substances. His results indicated that heat treatments of flour up to 170° F. increased starch resistance to diastase.

Recent investigations have shown that wheat starches from different sources vary in certain properties. Mangels (1926), and Hermano and Rask (1926) showed that wheat starches from different sources vary in their resistance to diastatic action. Malloch (1929) made a more extensive study of starch resistivity and found that variety, environment, and stage of maturity at cutting influenced the susceptibility of starches to diastatic action.

Mangels and Bailey (1933) have recently found considerable variation in viscosity or swelling capacity of wheat starches from different sources when treated with cold gelatinizing agents.

It may be presumed that different wheat starches would vary in their resistance to heat exposure. This paper presents data on the effect of exposure to heat on (1) specific rotation, (2) resistance to diastase, and (3) swelling capacity of the starches when treated with 0.10 molar NaOH.

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Starches

Source and Preparation

The starches used were prepared in the laboratory from the following types of patent flour: (1) Hard red spring, bleached; (2) hard red spring, unbleached; (3) hard red winter, bleached; (4) soft red winter, bleached; and (5) durum, unbleached. The method of preparation and purification of these starches will not be given in detail, but they were in all cases dried at room temperature and at no time during their preparation were they exposed to a temperature exceeding 30° C.

Heat Treatment of Starches

A Freas oven was used in making heat treatments of the starches. The desired quantity of the air-dry starch was weighed into a 200 cc. Erlenmeyer flask and the flask placed on the upper shelf of the oven. The thermometer bulb in the oven was on a level with the bottom of the flask. The starches were in all cases exposed to the desired temperature for a period of 8 hours.

Effect of Heat on Specific Rotation of Starches

The original starch was compared with starch exposed at 80°, 100°, and 120° C., respectively, for 8 hours. The rotating power of the starches was determined by dispersing in 1 + 1 HCl. No clarifying agent was necessary if polariscope readings were taken within 30 minutes after dispersion. The procedure used was as follows: The equivalent of 2 gms. of dry starch was weighed into a 200 cc. Erlenmeyer flask and 50 cc. of cold distilled water added. The flask was rotated to bring the starch in suspension, and then 50 cc. of concentrated HCl (sp. gr. 1.2) were added from a pipette. The flask was held in an ice-bath during the addition of the HCl and kept there until a temperature of 20° C. was reached. The clear solution was polarized in a 20 cm. tube. The specific rotations were calculated and are given in Table I.

TABLE I

Effect of Heating on Specific Rotation of Starches

Source of starch	Check not heated	Heated 8 hrs. at 80° C.	Heated 8 hrs. at 100° C.	Heated 8 hrs. at 120° C.
	αD	αD	αD	αD
Hard red spring, patent, bleached	201.3	200.4	201.3	200.3
Hard red spring, patent, unbleached	201.2	201.2	201.5	200.6
Hard red winter, patent, bleached	201.6	201.7	201.7	200.8
Soft red winter, patent, bleached	202.2	200.9	200.8	200.4
Durum, patent, unbleached	201.3	200.7	200.0	200.9
Average (5)	201.5	201.0	201.1	200.6

The specific rotation of starches is not greatly affected by heat. Starches heated 8 hours at 80°, 100°, and 120° C., respectively, do not show any marked change in specific rotation.

The general tendency of effect of heat exposure was to slightly decrease the specific rotation. The average specific rotation of the five original starches was 201.5; heated 8 hours at 80° C., 201.0; heated 8 hours at 100° C., 201.1; and when heated 8 hours at 120° C., 200.6. These changes, however, considering the dilute solutions used, are within the range of experimental error, and individual starches did not show consistent differences.

Effect of Heating on Resistance to Diastase

The original starch was compared with starch exposed 8 hours at 100° C. A solution prepared by suspending a hard red spring patent (5 gms. to 100 cc.) in a sodium acetate buffer solution (pH 4.6–4.8) and then centrifuging, was used as a source of diastase. The diastatic activity was determined by the method recently recommended by Blish (1933).

The data for the five starches are given in Table I, and show that prolonged heating at 100° C. decreases slightly the resistance of starch to diastatic attack. While the increase in maltose formation due to previous heating of the starch is small, it is quite consistent except in case of the starch from bleached hard red spring patent. The data in

TABLE II

Effect of Heating at 100° C. on Resistance to Diastase

Starch source	Source of diastase	Milligrams of maltose formed from 10 gms. of starch	
		Original starch	Heated at 100° C.
Hard red spring, patent, bleached	H.R.S. flour	31	32
Hard red spring, patent, unbleached	" "	41	45
Hard red winter, patent, bleached	" "	42	46
Soft red winter, patent, bleached	" "	27	31
Durum, patent, unbleached	" "	47	52

Table II would indicate that heat treatment of flour does not greatly affect susceptibility of starch to diastatic action.

Effect of Heat on Swelling Capacity or Viscosity

Exposures to 80°, 90°, and 100° C. were used for these studies. Some observations were also made at higher temperatures, but the results were erratic, and could not be consistently replicated. The pro-

cedure used in determining swelling capacity was as follows: The equivalent of 5 gms. of moisture-free starch was weighed into 200 cc. Erlenmeyer flasks. The starches were given the desired exposure to heat as previously described.

The flasks were then placed in a thermostat bath held at 30° C. and allowed to reach that temperature, after which 100 cc. of 0.1 molar NaOH at 30° C. was added to each flask. The starch and 0.1 molar NaOH were mixed thoroughly by rotating the flask and replaced in the thermostat. At the expiration of 1, 3, and 6 hours, the viscosity of the starch-NaOH suspension was determined by means of an Ostwald pipette. The method of determining viscosity is described in a recent paper by Mangels and Bailey (1933). Viscosity is reported as t/tw where t is the time of flow for the starch suspension and tw the time of flow for distilled water.

The starches were exposed for 8 hours at 80°, 90°, and 100° C., respectively, and a portion of the untreated starch was used as a check. The difference in swelling capacity as measured by viscosity is shown in Table III and Figures 1 to 6.

TABLE III

COMPARISON OF DIFFERENT STARCHES

5% Starch Suspensions in 0.1 Molar NaOH Solutions
Starches exposed to dry heat before adding NaOH Solution

Starch source	Duration of heating	Temperature	Viscosity $\frac{t}{tw}$ at 30° C.			
			Initial	1 hr.	3 hrs.	6 hrs.
	(*Hrs.*)	° *C.*				
Hard red spring, bleached	0	Check	1.1	1.4	2.7	4.1
	8	80	1.1	1.6	3.0	4.2
	8	90	1.1	1.7	3.6	4.9
	8	100	1.1	2.2	5.2	7.5
Hard red spring, unbleached	0	Check	1.1	1.3	2.1	2.7
	8	80	1.1	1.6	2.8	3.7
	8	90	1.1	1.9	3.8	5.4
	8	100	1.1	2.6	5.3	7.1
Hard red winter	0	Check	1.1	1.5	3.4	5.1
	8	80	1.1	2.3	5.9	8.4
	8	90	1.1	2.6	7.9	9.8
	8	100	1.1	4.6	15.0	19.8
Soft red winter	0	Check	1.1	1.3	2.6	4.0
	8	80	1.1	1.7	3.5	5.1
	8	90	1.1	2.1	5.6	8.7
	8	100	1.1	4.3	13.4	18.9
Durum	0	Check	1.1	2.4	9.4	15.8
	8	80	1.1	3.3	12.1	16.5
	8	90	1.1	5.1	15.1	22.4
	8	100	1.1	7.1	22.2	29.4

Figure 1 shows the relative viscosity of the five starches without heat treatment and includes data on a sample of commercial wheat starch. The order of viscosity for the untreated starches is (1) durum, (2) hard red winter, (3) soft red winter, (4) hard red spring, bleached, (5) hard red spring, unbleached, and (6) the commercial wheat starch.

While exposure of eight hours to 80°, 90°, and 100° C., respectively,

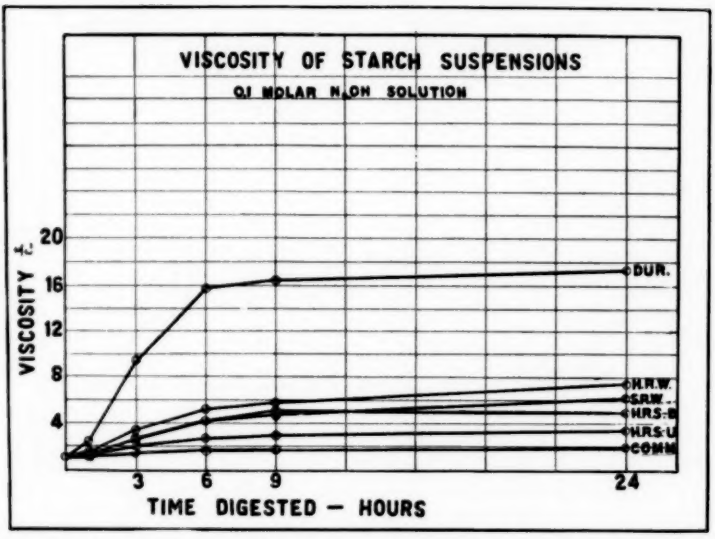

FIG. 1. Viscosity of starch suspensions of patent flours milled from various classes of wheat.

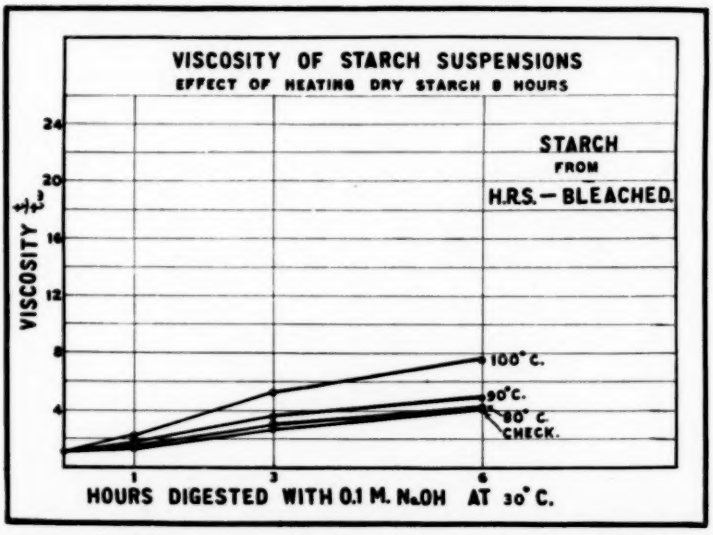

FIG. 2. Effect of heat exposure on viscosity of starch suspensions from a bleached hard red spring patent flour.

increases the viscosity of the hard red spring starch preparations, the magnitude of the change is relatively small compared with the change shown by the other starches. The unbleached hard red spring shows somewhat greater change than the starch from bleached flour.

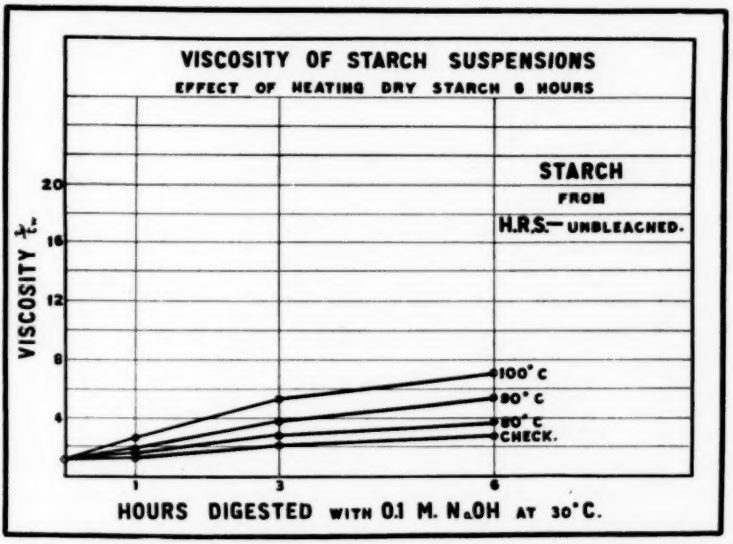

FIG. 3. Effect of heat exposure on viscosity of starch suspensions from an unbleached hard red spring patent flour.

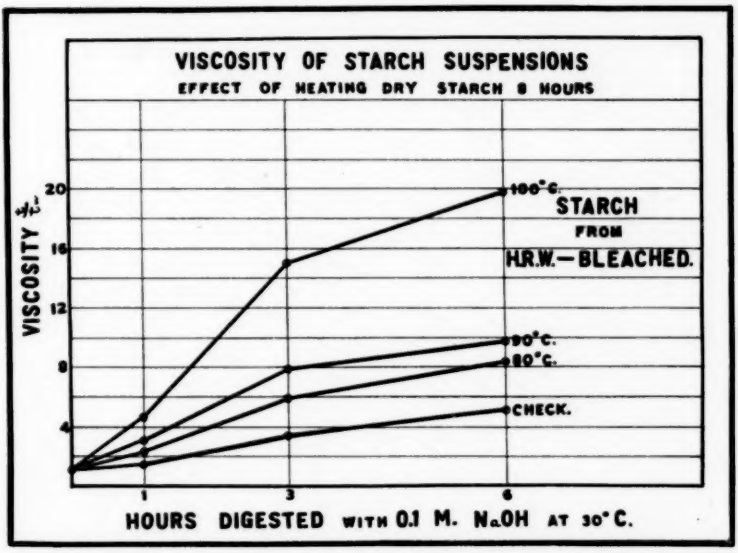

FIG. 4. Effect of heat exposure on viscosity of starch suspensions from a bleached hard red winter patent flour.

The hard red winter and the soft red winter starches show relatively much greater increase in viscosity than the hard red spring starches particularly when exposed at 100° C. Exposure at 80° and 90° C. does not exert as great a change relatively as exposure at 100° C.

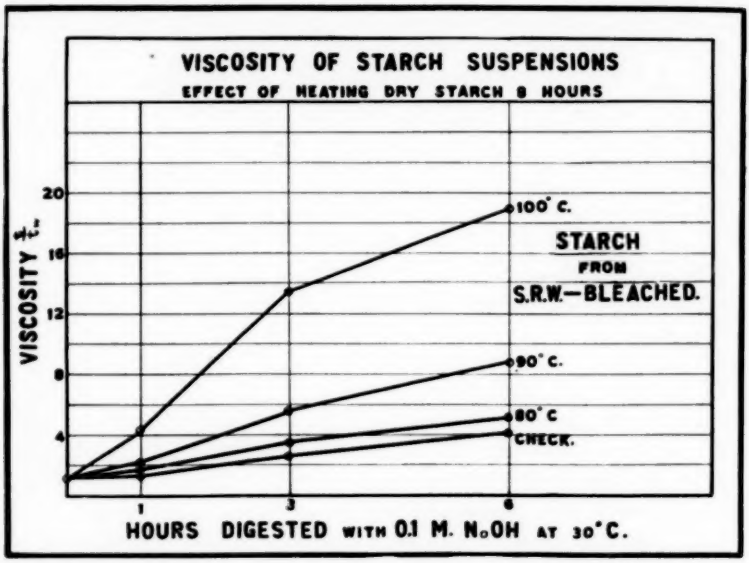

FIG. 5. Effect of heat exposure on viscosity of starch suspensions from a bleached soft red winter patent flour.

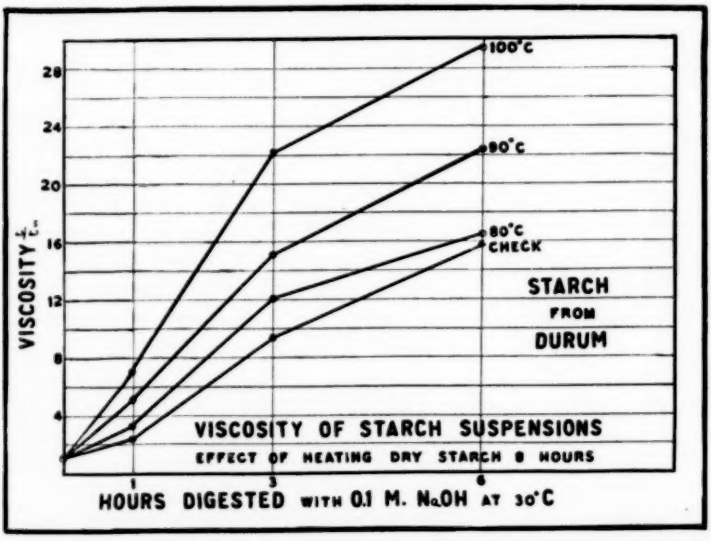

FIG. 6. Effect of heat exposure on viscosity of starch suspensions from an unbleached durum patent flour.

The durum starch shows about the same amount of increase due to exposure at 100° C. as the winter wheat starches, but the level of viscosity is higher because the initial swelling capacity of this starch is greater. Exposure at 90° C., however, has relatively more effect on the durum starch than on the winter wheat starches.

Rask and Alsberg (1924) have called attention to the similarity of the durum and hard winter wheat starches and points out that both of these types of wheat are more suitable for macaroni than hard red spring wheat.

Mangels and Bailey (1933) have recently pointed out the similarity of winter wheat starches and durum starches when treated with different cold gelatinizing agents.

The starches from these different types of wheat show interesting differences in reaction to heat. The winter wheat starches are quite similar in viscosity both in case of the untreated material and after exposure to heat. The reaction of the durum starch to heat is rather similar to that of the winter wheat starches. The spring wheat starches, however, are less sensitive to heat than the other types.

Summary

1. Exposure to 80°, 100°, and 120° C. for 8 hours has very little effect on the specific rotation of wheat starch, but there is a tendency for specific rotation to decrease.

2. Exposure to 100° C. for 8 hours decreased slightly the resistance of starch to diastase.

3. When starches are exposed to dry heat for 8 hours at 80°, 90°, and 100° C., respectively, the starch from hard red spring wheat flour shows relatively little change in swelling capacity.

4. The starches from winter wheats, when subjected to heat treatment, show a much greater increase in swelling capacity than starches from hard spring wheat similarly treated. Exposure at 80° or 90° C., however, gives relatively small changes as compared to exposure at 100° C. Durum starch shows about the same increase in viscosity as the winter wheat starches when exposed to heat.

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QUICK ASH METHODS[1]

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The determination of ash in cereal products is frequently thought of as an exact measure of their mineral content. A study of the facts readily indicates that the results are, on the contrary, somewhat empirical. In the case of flour ash, this is chiefly due to the fact that phosphoric acid is to a certain extent volatile even at the lowest practicable ashing temperatures, and most flours have a greater or less excess of phosphoric acid over metallic radicals. Variation in temperature of burning may also make some change in the equilibrium between the carbonates and the oxides of the alkaline earth metals.

A difficulty of somewhat different nature arises from the fact that if the temperature of burning is high enough to fuse part of the ash, the fused material is likely to enclose particles of carbon and protect them from being burned. This is particularly troublesome when appreciable amounts of silica are present, since a glass of low melting point may be formed.

Thus, the commonly accepted method for the flour-ash determination is to burn at a temperature of about 550° C. until the carbon is completely oxidized, or nearly so. In the case of flour, this ordinarily requires from 5 to 7 hours, and sometimes even longer, partly due to the fact that, when burning starts, the flour shrinks together into a compact char into which air penetrates slowly.

The difficulty with this method of determining ash as a means of mill control is evident when it is considered that a mill may produce carloads of off-grade flour before the results of the ash determination

[1] Contribution No. 46, Department of Milling Industry.

are available. Numerous methods have been devised for hastening the burning of the flour. Some of these are discussed by Walters.[2] One of the older devices was to moisten the flour with a measured amount of an aqueous solution of calcium acetate, and burn at a high temperature. The excess of calcium prevents both the volatilization of the phosphoric acid and the fusion of the ash. An additional advantage is that the char does not shrink so much, but remains porous so that air can more readily enter. Difficulties encountered in the method are the errors in measuring the calcium acetate solution, the hygroscopic nature of the ash produced, and the slow careful drying necessary before burning the sample. It is probably also true that samples containing a considerable excess of phosphoric acid over basic elements will give higher results by the use of calcium acetate than by the official method.

Two methods which depend solely on preventing the shrinking of the flour char are the glycerol-alcohol method and the powdered alundum method. Both have given good results, but usually require considerably over an hour, and have not gained much favor.

Recently a method has been described [3] using an alcoholic solution of magnesium acetate. By using a dilute solution, the error in measuring is kept small. The samples are burned at 850° C., requiring about 45 minutes. One considerable drawback to the method is the high muffle temperature, which makes it almost necessary to have a separate muffle for determining ash by the official method, which is necessary for determining the blank of the solution used.

Some laboratories pipe a supply of oxygen to the muffle to hasten burning, sometimes using it in addition to other aids mentioned.

In 1928 and 1929 an attempt was made in this laboratory to overcome the more serious drawbacks of the calcium acetate method. Magnesium nitrate was substituted for the calcium acetate because the nitrate aided in the combustion and the magnesium gave an ash that was less hygroscopic than when calcium was used. Alcohol was used as a solvent for the magnesium nitrate since it is quickly absorbed by the flour and no stirring is required to wet all the flour; and the strength of the solution was so adjusted that when just enough was used to completely moisten the flour, the weight of the resulting ash was just slightly less than doubled. In arriving at this concentration three factors were taken into consideration. First, it is desirable that there be an excess of magnesium over any possible content of phosphoric acid. This becomes absolutely necessary if temperatures higher than

[2] Walters, F. M. New aids in ashing flour. 1930. Cereal Chem. **7**: 83–87.

[3] Davis, C. F. Report of collaborative studies on the magnesium-acetate-alcohol quick ash procedure. 1932. Cereal Chem. **9**: 431–437.

550° C. are used for ashing, in which case a phosphated flour would require a solution of higher magnesium content. Second, the greater the amount of magnesium used, within reasonable limits, the more porous the char produced, and therefore the more rapid the burning. Third, the greater the amount of magnesium used, the greater the error due to inaccuracies in measuring the solution to be added to each charge. Obviously a point of compromise must be found between these last two, and therefore the solution strength chosen may depend somewhat upon the burning speed desired, the accuracy possible in measuring the solution, and the allowable variation in ash results.

In 1930 John A. Shellenberger, a graduate student, proposed a micro ash method, and while no micro balance was available some experimentation showed that a tenth-gram sample burned in about an hour. Two years later Wm. Ainsworth and Sons made the department the loan of an assay balance for the continuation of this work. This balance, with a capacity of only 1 gram, has a very light beam and hangers, and so combines the required sensitivity with the speed and stability of a good analytical balance, and is even less disturbed by mill vibration. By the use of a half-milligram rider, a weight of .005 mg. is read directly from the beam.

For burning the ash, platinum dishes were made similar to the standard No. 1 platinum crucible cover, but with the depression extending nearly to the edge so that only a very narrow rim is left. For the 0.3 gm. flour samples finally adopted, a larger dish would be better, such as a No. 2 or a No. 3 crucible cover. In using magnesium nitrate solution with these small samples it was found that when 95% alcohol was used the ash was very fluffy and tended to blow away, while if 50% alcohol was used, the sample was slow to burn, and required greater care in drying before placing in the muffle. Both difficulties were overcome by the use of 70% alcohol.

The following tables show the results obtained from five representative hard winter wheat flour samples. In all cases the blank was determined by burning a sixth flour which gave an ash content of 0.456% by the official method. In the preliminary trials shown in Table I, the magnesium nitrate solution was measured onto the flour by merely counting ten drops. The results are, of course, highly variable, especially with the more concentrated solution, the purpose being merely to practice on details of technique.

It was evident that the use of 0.1 gm. sample necessitated weighing the ash accurately to the finest subdivision on the balance rider. With any balance this increases the time required in weighing, so 0.3 gm. samples were used in all further trials, and a micropipette was con-

TABLE I

PRELIMINARY TRIALS WITHOUT ACCURATE MEASUREMENT OF MAGNESIUM NITRATE SOLUTION

Flour sample	Size of sample	Ash as weighed	Blank	Ash	Ash by official method
	Gm.	*Gm.*	*Gm.*	*P.ct.*	*P.ct.*
A	0.1	0.00107	0.000614	0.456	0.450
		.00105		.436	
		.00102		.406	
B	0.1	0.00105	0.000614	0.436	0.432
		.00110		.486	
		.00104		.426	
A	0.1	0.00080	0.000339	0.461	0.450
		.00080		.461	
		.00079		.451	
B	0.1	0.00077	0.000339	0.431	0.432
		.00077		.431	
		.00078		.441	
C	0.1	0.00083	0.000339	0.491	0.466
		.00081		.471	
		.00080		.461	

TABLE II

EFFECT OF DIFFERENT CONCENTRATIONS OF MAGNESIUM NITRATE SOLUTIONS AS WELL AS INCREASE IN THE SIZE OF THE ASHING CHARGE ON THE ACCURACY OF THE ASH DETERMINATION

Flour sample	Size of sample	Ash as weighed	Blank	Difference	Ash	Ash by official method
	Gm.	*Gm.*	*Gm.*	*Gm.*	*P.ct.*	*P.ct.*
A	0.3	0.002500	0.001142	0.001358	0.453	0.450
		.002505		.001363	.454	
		.002495		.001353	.451	
B	0.3	0.002410	0.001142	0.001268	0.423	0.432
		.002450		.001308	.436	
		.002440		.001298	.433	
C	0.3	0.002565	0.001142	0.001208	0.474	0.466
		.002530		.001388	.463	
		.002535		.001393	.464	
D	0.3	0.002350	0.001142	0.001208	0.403	0.404
		.002350		.001208	.403	
		.002360		.001218	.406	
E	0.3	0.002780	0.001142	0.001638	0.546	0.544
		.002775		.001633	.544	
		.002785		.001643	.548	
D	0.3	0.00303	0.001823	0.001207	0.402	0.404
		.00305		.001227	.409	
		.00302		.001197	.399	
E	0.3	0.00344	0.001823	0.001617	0.539	0.544
		.00346		.001637	.546	
		.00346		.001637	.546	

structed which delivered 0.37 cc. of magnesium nitrate solution. The results obtained are shown in Table II, using two different concentrations of solution on two of the samples.

The time required for burning is influenced by the size of the sample used as well as by the concentration of the magnesium nitrate solution added. The 0.1 gm. samples burned within 5 minutes when the more concentrated solution was used, and required 8 to 10 minutes with the weaker solution. The 0.3 gm. samples required 7 or 8 minutes burning with the more concentrated solution and about 15 minutes with weaker solution.

The following procedure appeared to give the most satisfactory results:

Three-tenths of a gram of flour is weighed in a light aluminum scoop, transferred to the platinum dish and flattened out by tapping. By means of a micro pipette 0.35 cc. of a solution containing 10 gms. of crystalline magnesium nitrate in 500 cc. of 70% alcohol is dropped into the center of the flour. The micro pipette may easily be drawn from small-bore tubing and marked to deliver any amount between 0.35 and 0.39 cc. As soon as the solution has completely penetrated, it is ignited with a match, during which process the dish should rest on a cold plate of porcelain, metal or other material that will prevent it from becoming hot enough to cause spattering. As soon as the alcohol is burned off the dish is placed in a muffle at 550° C., and burning is usually complete in from 5 to 10 minutes. The dish is placed in a desiccator and cooling is complete by the time the desiccator can be carried to the balance. The ash is transferred to the balance pan by merely tapping the dish and weighed directly. The blank of the magnesium nitrate solution is determined by determining ash on one or two samples by the ordinary method, or by burning a 0.3 gm. sample without using the solution.

Conclusions

Three advantages are to be secured by this method: (1) The platinum dishes used are very light and inexpensive, (2) the low burning temperature used gives a longer muffle life and allows determination by the official method in the same muffle, and (3), most important of all, the burning time is very short. More rapid burning could be obtained by increasing the muffle temperature, but with the flours used in these tests this has not seemed desirable.

The time used in handling the ash dishes can be reduced by placing them on a silica plate about one-eighth of an inch thick. When the preliminary burning is complete the entire plate is transferred to the muffle, using a handle of chrome nickel steel which fits into a hole drilled in one edge of the plate. Only 3 to 5 minutes longer time is required in the muffle, and the saving in handling is considerable.

REPORT OF THE CEREAL SECTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

J. A. LeClerc, *General Referee on Cereal Products, 1932*

Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.

(Received for publication December 2, 1933)

The Cereal Section of the Association of Official Agricultural Chemists held its annual meeting at the Raleigh Hotel, Washington, D. C., on November 7, 1933. This was the 49th annual meeting of the Association. In the Cereal Section alone there were present over 50 chemists representing a splendid cross-section of the milling, baking, and allied trade industries as well as institutions of learning both of this country and of Canada. The General Referee, who acted as chairman, called attention to the desirability of standardizing the methods used by chemists of the various countries for the purpose of evaluating wheat, flour, and flour products. This same subject was discussed by the cereal chemists who attended the First International Congress of Panification, held in Rome, June 1932, at which time favorable action was taken on a motion to study ways and means of bringing this about. It is a fact to be deplored that this country, which boasts of a billion dollar milling industry and a billion dollar baking industry and which has cereal chemists in various Government bureaus and in numerous State Institutions, did not have a single representative at the Congress. Delegates from five South American countries as well as from Canada comingled with similar delegations from seventeen European countries, and heard and discussed reports of prime interest to the various industries connected with Cereals.

The Chairman called attention to the desirability of enlarging the scope of the Section's activities (as the result of the repeal of the 18th Amendment) by making a more intensive study of methods for the analysis and evaluation of corn, rice, and barley products used by the brewing and distilling industries. He further called upon those present (and he makes this same request to those who may read this report) to indicate how the activities of this Section may be enlarged and/or improved so as to be of greatest good to the cereal industries. Besides the association referee's reports, giving the results of collaboration, three special papers were presented.

Dr. H. E. Barnard's paper was entitled, " Products of Corn," and included a popular description of the various parts of the corn kernel and the uses to which the products obtained from the germ, hull, and endosperm are put; the history of starch, dextrine, corn syrup and corn sugar manufacture, and statistics regarding the industrial utilization of the products made from corn starch. The speaker also referred to lactic acid, one of the newer products made from starch, and its uses in the arts and industry.

Dr. C. B. Morison, of the American Institute of Baking, spoke on, " Some Problems of Interest to the Baking Industry." In this he discussed the kind of chemist that the baker needs today. He stated in part, " . . . that scientific and technical problems are found along the line of production from ingredients to products. These problems are best appreciated by the chemist who has supplemented his training in science by actual experience in bakery production under the guidance of an expert practical baker. However, bakers cannot function as chemists, nor should chemists endeavor to become bakers. A mutual interest in the objective is necessary, that is, progress in the direction of a better understanding of an art which may become more ' scientific ' in the future."

Reference was made to current ideas in nutrition as they relate to bread, and some of the " fashions " of the cereal chemists. The chemist in baking should always remember that the baker's confidence in the chemist depends upon the latter's ability to make science " work."

Dr. Charles N. Frey of the Fleischmann Laboratories, New York City, read a highly scientific paper on " Enzymes of Flour in Relation to Yeast Fermentation." It was pointed out by Dr. Frey that diastatic activity in bread production was recognized by Wood as well as by Humphries in 1907. Rumsey in 1922 showed that autolytic diastatic activity (saccharogenic activity) is an important factor in the baking industry. The dual nature of autolysis was recognized by Stone in 1898; Whymper, 1907; Dox and contemporaries in 1916. The work of Alsberg, 1927, and Mangels, 1928, confirmed the results of the previous workers. Kent-Jones recognized the environmental nature of saccharogenic activity in 1925. Halton and Fisher, 1928; Jørgenson, 1929; and Swanson and Kroeker, 1932, confirmed this view.

Dough fermentation is conditioned by two independent enzymic systems: (*A*) Diastatic enzymes of flour; and (*B*) Yeast enzymes, invertase, maltase, and the zymase system.

Diastatic activity may be designated as a logarithmic function of the time, whereas zymatic activity is essentially a linear function of the time.

Among the reports of the Referees were the following:

(1) Color in Flour, *D. A. Coleman* (read by M. R. Coe). There

are at least four different devices for measuring color in flour either directly or in the extract. The first classification includes such devices as that of (1) Munsell, (2) Wallace & Tiernan, (3) Bausch & Lomb; the second class makes use of the spectrophotometer (Ferrari & Bailey) or the colorimeter modified after Winton.

The spectrophotometer, while accurate, is expensive; the colorimeter while inexpensive is not sufficiently accurate. Instruments that fall within the first category appear therefore more promising for this work.

(2) H–Ion Concentration of Flour, *R. J. Clark* (read by Emily Grewe). The procedure for the colorimetric determination of pH in bread is practically the same as that for flour. The Referee recommended the following for collaborative study.

"That Gamma (2:5) Dinitrophenol, range pH 4.0–5.6, and Paranitrophenol, range pH 5.4–7.0, be used in making colorimetric pH determinations on flour; and, that since the color change of both these indicators is from colorless to deep yellow, that a blue glass be placed over the slots on the comparator block opposite the etched glass to facilitate the readings."

(3) Diastatic Value of Flour, *M. J. Blish* (read by F. L. Dunlap). This is an elaborate collaborative study and reflects much credit on the Referee. The ferricyanide reduction method of Hagedorn and Jensen which is a modification of the Rumsey type of procedure was adopted as "Tentative" by the Association and will be published in full in the Proceedings.

(4) The Determination of Starch in Flour, *by V. E. Munsey.* The so-called Rask method which has been tested for several years, as modified by the Associate Referee, was made Tentative.

(5) Flour Bleaching Chemicals, *Dorothy B. Scott.* A comparative study was made of the modification of the present Tentative Method (Methods of Analysis, XX, 39) for Chlorine Determination with the method proposed by Kent-Jones (J. Soc. Chem. Ind., May 16, 1930, Vol. LXIX, No. 20, p. 223T). In all cases more chlorine was found by the former method than by the latter.

(6) Determination of Moisture in Air Dried Baked Products Other Than Bread, *by L. H. Bailey.* Fig bars and soda crackers were the products tested collaboratively, the moisture being determined by both the vacuum-oven and by the 130° air-oven methods. In the case of fig bars the results were not satisfactory and it was proposed to carry on further work.

(7) Determination of Crude Fiber in Baked Products, *by Ruth G. Capen.* Collaborative tests were carried on with crackers and fig bars. The results show that the method now official for feeds (Method of Analysis, XXVII, 17) is applicable for baked products.

(8) MILK SOLIDS IN MILK BREAD, *by Arnold H. Johnson* (read by B. D. Ingels). The method which appears to be the most promising is based upon the estimation of lactose. The crumb of the bread was used and extracted with ether. Sugars other than lactose were fermented with yeast. The lactose was then determined by Fehling's solution.

(9) RYE IN FLOUR MIXTURES, *J. H. Bornmann* (read by L. H. Bailey). The methods tested were (1) Tillmans' (modified), König-Bartschat's and the Chloroform procedure. The detection of rye qualitatively can be successfully made by the $CHCl_3$ method. Encouraging results were also obtained for the estimation of the quantity of rye, by the modified Tillmans' method (Z. unters. Lebens 63, p. 514, 1932).

(10) EXPERIMENTAL BAKING TEST, *by C. G. Harrel* (read by Clinton L. Brooke). This was an elaborate report of a study made to determine the importance of (*a*) dough temperature, (*b*) yeast quality. The report was profusely illustrated with photographs of loaves baked under the different conditions of the experiment. The conclusions follow:

Dough temperature.

1. "The findings of Merritt, Blish, and Sandstedt, Treloar and Larmour, and others—that a temperature range of $30 \pm 2.0°$ C. of the dough coming from the mixer is of no measurable influence on the 'pup' loaf baking test—are confirmed.

2. "The temperature adjustment of a 160-gram dough to the cabinet temperature proceeds much faster than that of a 480-gram dough with a range of $\pm 2.0°$ from the equilibrium temperature; about 40 minutes are necessary for a 160-gram dough and 90 to 100 minutes for a pound-loaf dough to bring the temperature of the dough within $\pm 0.5°$ C. of the equilibrium temperature.

3. "The equilibrium temperature in a 160-gram dough is 0.5° to 0.8° C. above the cabinet temperature (30° C.); that of a 480-gram dough is somewhat higher (1.0 to 1.5° C.).

4. "For the 'pup' loaf baking test, it is recommended that the fermentation cabinet temperature be set at 29.5° in order to assure a dough temperature of 30° C.

Yeast variability.

1. "Yeast can be stored for 2 to 3 weeks without any deleterious effect, provided it is guarded against higher temperature. As a safeguard against improper delivery and storage of yeast, we recommend the following specification: By 'fresh' yeast is meant yeast which is delivered at a temperature not higher than 15° C. and stored at a temperature not higher than 10° C. for a period not longer than 2 weeks.

2. " Large differences exist between yeasts of different brands or strains in regard to gas produced as well as effect on dough properties.

3. " The differences in regard to dough properties are largest in weaker flours and in doughs which are not properly developed in the mixing operation.

4. " Specifications for yeast in regard to gas production and influence on dough properties cannot be made, as different flours are not affected alike.

5. " It is therefore recommended that the suggestion made by Cook and Malloch (1930)—that arrangements be made with a yeast company to place a special yeast for experimental purposes on the market—be further studied."

No reports were received from Associate Referees regarding the following subjects: (1) Ash in flour, macaroni and baked products, (2) Foreign methods of testing flours, (3) CO_2 in self-rising flour, (4) Unsaponifiable matter in flour and baked products, (5) Lipoids, P_2O_5, fat and crude albumin N in flour and baked products.

The following changes in the status of the methods were adopted by the Association:

" That the method proposed by the associate referee for the determination of the diastatic power of flour be adopted as tentative.

" That the present tentative method (Method of Analysis, 1930, p. 172, paragraph 39) for the determination of starch be revised as outlined in the associate referee's report for 1932 and that the method be further studied.

" That the $CHCl_3$ test for the detection of rye flour in wheat flour be adopted as tentative.

" That the vacuum-oven method (official, first action) for the determination of H_2O in air-dried baked products (not containing fruit) other than bread be made official (final).

" That the 130° C. air-oven method (official, first action) for the determination of H_2O in air-dried baked products (not containing fruit) other than bread be made official (final).

" That the tentative method for the determination of crude fiber in baked products other than bread be made official, first action.

" That the method for the preparation of samples of macaroni products be retained as tentative and that work on this subject be temporarily discontinued."

The following recommendations were made regarding future activities:

" That special (non-collaborative) studies be conducted on the methods for the determination of unsaponifiable matter in the fat of flour,

baked products, and macaroni products (formerly called alimentary pastes), in conjunction with the same study on eggs.

"That further study be made of the tentative method for the determination of crude albumin nitrogen in flour, macaroni, and baked products, in conjunction with similar methods for eggs.

"That comparative tests be made of foreign and domestic methods of chemical analysis used as measures of evaluating flour, macaroni, and baked-products.

"That (2:5) dinitrophenol (pH = 4.0–5.6) and paranitrophenol (pH = 5.4–7.0) be studied collaboratively in making colorimetric determinations on flour, bread, and macaroni products using as a basic method the modification of the tentative method as suggested by the Associate Referee.

"That further study be made of the method of ashing flour, macaroni, and baked products with the object of reducing the time of combustion.

"That the acid hydrolysis method for the determination of fat in macaroni, and baked products, be further studied.

"That the method modified by the associate referee last year for the determination of phosphorus in eggs be studied collaboratively regarding its applicability to flour, macaroni- and baked-products.

"That the method as modified by the associate referee last year for the determination of lipoids and lipoid-phosphorus in flour, macaroni- and baked-products be further studied.

"That the methods for the determination of Cl in bleached flour, and that the method of the Associate Referee for the determination of benzoyl peroxide as benzoic acid in bleached flour be further studied.

"That the study of methods for the determination of color in flour be resumed, special attention being paid to the use of the Munsell or similar apparatus.

"That the $CHCl_3$ test, the modified Tillmans method, and the König-Bartschat method be further studied.

"That methods for the determination of CO_2 in self-rising flour be further studied.

"That methods for moisture in baked products containing fruit be further studied.

"That study be made of methods to determine milk solids in bread.

"That the method for the determination of chlorides in flour and baked products be further studied.

"That methods of determining chlorides in baked products (official, first action) be further studied before final action is taken.

"That a study be made of the methods and of the viscosimeter best

adapted for the determination of viscosity in flour-and-water-suspensions.

"That a study be made of the method for cold water-soluble extract of flour and at the same time the possibility of using this extract for the determination of acidity in flour.

"That a study be made of the methods for the determination of ergot in wheat and in rye flour.

"That a study be made of the methods for the determination of catalase, and proteolytic activity of flour."

CHEMISTRY OF CORN SEED GERMINATION

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(Received for publication June 13, 1933)

Introduction

A large number of chemical changes take place during corn germination. So, to the writer's knowledge, no systematical investigation of them has yet been reported, although Sachs (1887), Peters (1861), Fleury (1865), Müntz (1871), Schütenberger (1882), Green and Jackson (1906), Eckerson (1913), Choate (1921), Maquenne (1898), Schertz (1920), Atkinson (1922), Brown and Escombe (1898), Brown and Morris (1890), Bruschi (1908), and others have reported some chemical aspects of several seeds.

The present experiments were designed to find the chemical and calorific changes which take place during germination of corn seeds and their *attached* and *isolated* embryos and endosperms. It has been stated in almost all texts that a seedling grows at the expense of endospermic food reserves.

Experimental

Uniform corn seeds were divided into five duplicate lots of 200 each, after Malhotra's (1931) procedure. Except one (control), all lots were allowed to germinate for 2, 4, 6, and 8 days, following the techniques of Malhotra (1930). At the specified time of each lot the seedlings were heated at 110° C. for one hour to kill enzymes, dried to constant weight at 80° C., cooled in desiccators, pulverized to pass a 60-mesh screen, according to the author's method. Controls were similarly treated.

Fats were extracted with petrolic ether. The residue was treated with 60% ethyl alcohol in order to extract sugars, which were determined

by the Munson and Walker method as recorded by Mathews (1931). Saliva was added to the residue in order to reduce starch into sugars. The latter were determined as before. Hemicelluloses were estimated according to Malhotra (1931a).

Total nitrogen was determined by Arnold-Gunning's modification of Kjeldahl's method. Ash was estimated by completely burning the samples in an electric muffle. Results have been expressed in percentages of dry weight.

Calories of heat were determined by the Parr oxygen bomb calorimeter. Directions and precautions laid down by the Central Scientific Company, Chicago, were carefully followed. Appropriate corrections were made. All analyses were carried out in duplicate.

Germination and chemical analyses were repeated. Corn embryo and endosperm rather than seeds were utilized this time. These experiments were repeated again employing previously isolated embryo and endosperm for 12 days. Typical corn embryos and endosperms are shown in Figure 1.

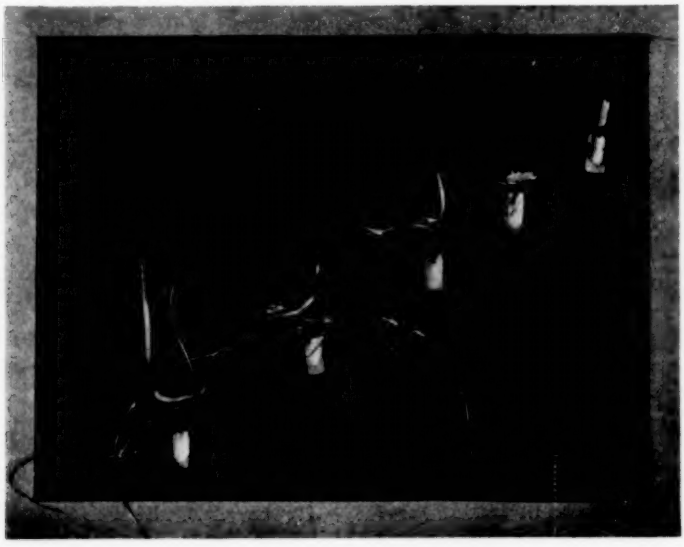

Fig. 1. Typical corn embryos and endosperms at various stages of germination. From right to left: Control, germination after 2, 4, 8, and 10 days, respectively. Upper row, embryos; lower, endosperms.

Results

Fats, dry weight, starch, hemicellulose, and calories of heat decreased while sugars increased. Constituents of corn seed have been presented in Table I. Ash and total nitrogen remained constant, although it ap-

TABLE I

DISTRIBUTION OF RESERVE MATERIALS[1] AND HEAT ENERGY IN CORN SEEDS DURING GERMINATION

Treatment	Fats	Loss in dry weight	Ash	Sugars	Starch	Hemicellulose	Total nitrogen	Calories of heat per gram
	P.ct.	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	
Ungerminated	4.88	0.0	1.30	2.5	27.5	30.7	1.7	3,480
Germinated 2 days	4.71	10.8	1.42	5.2	23.2	19.9	1.5	3,458
Germinated 4 days	4.60	11.6	1.42	10.2	21.1	16.1	1.4	3,428
Germinated 6 days	4.40	11.3	1.45	8.6	20.0	11.6	1.2	3,414
Germinated 8 days	4.09	12.2	1.42	15.6	18.1	11.1	1.7	3,301

[1] Results as percentages of dry weight.

pears that ash increased during germination, because a number of organic constituents decreased.

Data for chemical changes in *attached* embryos and endosperms (until the germination was discontinued) have been recorded in Table II. Attached embryo was rich in fats, sugars, nitrogen, and calories of heat; while endosperm contained more starch and hemicelluloses.

TABLE II

DISTRIBUTION OF RESERVE MATERIALS[1] IN ATTACHED EMBRYO AND ENDOSPERM DURING GERMINATION

Treatment	Ash	Fats	Sugars	Starch	Hemicelluloses	Total nitrogen	Calories of heat per gram
	P.ct.	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	
Endosperm							
Ungerminated	0.56	0.93	7.3	38.0	19.6	1.3	3,392
Germinated 2 days	0.56	1.40	13.2	36.2	13.9	1.7	3,352
Germinated 4 days	0.47	1.38	4.2	34.4	10.0	1.1	3,314
Germinated 8 days	1.03	1.15	9.1	26.2	9.5	1.7	3,302
Germinated 10 days	0.80	1.56	6.0	20.8	9.0	1.5	3,280
Embryo							
Ungerminated	8.74	30.0	14.8	5.5	10.8	3.7	4,415
Germinated 2 days	11.99	28.82	13.4	4.7	10.5	3.5	4,350
Germinated 4 days	9.13	27.23	8.9	4.7	10.7	3.5	4,034
Germinated 8 days	9.92	19.0	13.0	4.8	10.4	2.8	4,005
Germinated 10 days	8.37	17.1	12.8	4.4	9.3	2.4	3,968

[1] Results as percentages of dry weight.

Ash remained constant in endosperm, while it increased, decreased, and finally increased again in embryo. Fat was transferred from embryo to endosperm. Some fat was used up in the germination process. Sugar did not show any definite trend. Starch, hemicelluloses, and calories of heat decreased. No diminution was noted in nitrogen although it was transferred from one tissue to another. Data for chemical

changes in isolated embryo and endosperm have been presented in Table III. Practically no change took place in the isolated endosperm, except

TABLE III

DISTRIBUTION OF RESERVE MATERIALS[1] AND ENERGY IN ISOLATED EMBRYOS AND ENDOSPERMS DURING GERMINATION

Treatment	Fats	Ash	Nitrogen	Sugars	Starch	Hemicelluloses	Calories of heat per gram
	P.ct.	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	*P.ct.*	
Embryo							
Ungerminated	28.5	8.7	2.4	15.6	21.0	16.7	4,350
Germinated 2 days	21.5	6.8	3.0	17.2	32.7	16.4	3,920
Germinated 4 days	15.7	8.0	5.1	18.3	28.2	14.0	3,920
Germinated 8 days	10.4	7.5	5.6	18.9	24.6	13.1	3,682
Germinated 12 days	8.0	9.1	2.1	26.4	3.8	11.3	3,207
Endosperm							
Ungerminated	1.1	0.60	1.3	7.0	38.8	19.2	3,327
Germinated 2 days	1.0	0.61	1.3	13.2	31.6	19.1	3,311
Germinated 4 days	0.98	0.60	1.3	15.4	29.1	19.2	3,300
Germinated 8 days	0.95	0.60	1.3	15.9	28.4	19.0	3,279
Germinated 12 days	0.92	0.59	1.1	18.8	24.7	19.0	3,262

[1] Results as percentages of dry weight.

in sugar and starch. The former increased while the latter decreased because of the hydrolytic action of enzymes. Since endosperm alone is not capable of growth, no decrease in chemical ingredients was evident.

In isolated embryo, on the other hand, fat was utilized after transformation into starch and finally into sugars. Hemicelluloses and calories of heat also decreased. Murneek (1929) has already shown the importance of hemicelluloses as usable substances of the protoplasm. The remaining substances increased.

Summary

Starch, hemicelluloses, calories of heat, as well as fats, decreased, while sugars increased during corn seed germination. Proteins remained constant. Seedling weighed less than seed. Embryo contributed fats, and endosperm supplied carbohydrates during germination. When corn isolated embryo was allowed to germinate, fats, starch, hemicelluloses, sugar, and calories of heat decreased. Nitrogen and ash did not change. Chemical substances did not change in germinating embryoless endosperm, although starch was reduced to sugar by auto-digestion.

The work was carried out in the Biological Department of Saint Mary's College, St. Mary, Kansas.

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PRACTICAL OBSERVATIONS ON BREAD AND CRACKER FLOURS

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(Read at the Convention, June, 1933)

The selection of flour for bread and crackers requires special consideration for the following reasons:

1. It is the major ingredient used, 86.16% in bread and 89.77% in crackers (see Table I), and in itself it is practically responsible for the general character of the finished product.
2. It is the most troublesome ingredient from a standpoint of uniformity, due mainly to the variations in quality of wheat.

Although it is very well understood that hard spring and hard winter wheats are used for bread baking, and soft winter wheat in the manufacture of crackers, yet, probably due to the fact that both types of flour are subjected to yeast fermentation, it appears to be a general idea that the characteristics of cracker flour should be similar to those of bread flour. This is not the case, and the object of this paper is to place before you a comparison of the two products.

There are certain desired characteristics which are similar in both bread and cracker flours, namely, (1) general appearance and flavor as related to the finished product; and (2) working-quality from a standpoint of high-speed, commercial production.

The aim of the bread and cracker baker is to produce products which appeal both to the eye and taste of the consuming public. From a commercial standpoint it is essential to use flours that will operate satisfactorily, especially so under the present high-speed production schedules. Bread bakers, in recent years, have experienced difficulty with tough, bucky doughs; and cracker bakers are often troubled with short doughs. In such instances the trouble cannot be remedied by any machine adjustment, as the trouble is due to certain flour characteristics inherent in the wheat itself and which do not usually show up in the ordinary laboratory tests. These conditions have become more apparent with the introduction of high-speed production methods, and are problems that remain for solution.

The following are certain characteristics which are very dissimilar in bread and cracker flours:

(*a*) Color
(*b*) Absorption
(*c*) Fermentation.

Color (as related to inside and outside color of the finished product): The bread baker is interested in color of crumb and selects flour of such color character as to meet this requirement. There is not so much concern about crust color (bloom) because lack of it may easily be corrected by the addition of sugar or sugar-forming enzyme material. *In other words, in bread baking the color of crumb is governed by flour, and crust color by added ingredients as well.*

On the other hand, in crackers the inside color does not vary a great deal with different grades of flour. This is partly due to the small surface area (thinness) of a cracker, but it has also been proven through commercial tests that because of long fermentation time (Table I) the inside color is to a great extent equalized. It was further found that variations in the inside color of crackers, if any, is usually due to the use of excess baking soda ($NaHCO_3$).

The particular concern of the cracker baker is the outside color (bloom) of the finished product. The present day high-speed production, with its fast bake at high temperatures, tends to produce a product that is pale and lacking in natural bloom. The practice of bread bakers in using sugar-containing-materials to secure bloom is not satisfactory to the cracker baker, inasmuch as sugar imparts sweetness and moisture-retaining properties which are very undesirable in crackers. It is likewise undesirable to add an excess of baking soda for the reason that it imparts an unnatural yellowish-green color and also affects the flavor of the product. *Consequently, the cracker baker must rely mainly on flour to secure the desired bloom.*

Absorption: It is a well known fact that bread bakers prefer flour with high absorption qualities for the purpose of production yield. Flour which takes more water in mixing, and retains it, is desirable. Likewise it is desirable in bread baking to use other ingredients that increase absorption and moisture retaining power.

Just the opposite is true in cracker baking. The absorption qualities of flour should be low. A light, dry, crisp product is desirable and any extra water added must be evaporated in the oven. Flour with high absorption usually gives a solid, heavy, and tough product, instead of a light, crisp one.

Fermentation: In comparing the figures given in Table I, it can be seen that the cracker formula contains less water, less yeast, no sugar or

sugar-forming enzyme material, no milk, and that the shortening is in such quantity that it retards fermentation. Also it can be seen, that the flour represents 56.83% of the total bread batch as compared with 69.26% of the total cracker batch.

In bread doughs, all conditions are made favorable to obtain optimum results in fermentation, and natural deficiencies in flour may be remedied to some extent by liberal addition of added ingredients. On the contrary in cracker doughs, the use of added ingredients, due to long fermentation time (Table I), is very limited and the flour itself must produce necessary fermentative material.

The natural ability of a flour to respond to fermentation is much more important in crackers than in bread. *In bread, healthy fermentation is produced by flour and added ingredients; in crackers flour alone is responsible.*

TABLE I

COMMERCIAL FORMULAS FOR BREAD AND CRACKER DOUGHS
(*Considered as Average by the Writer*)

Dough ingredients	Bread	Cracker
	P.ct.	*P.ct.*
Flour	100.00	100.00
Yeast	1.50	0.30
Yeast food	0.30	—
Salt	2.00	1.00
Sugar (sucrose)	3.50	—
Malt extract	0.75	—
Milk solids	5.00	—
Shortening	3.00	9.50
Baking soda	—	0.60
Total dry ingredients	116.05	111.40
Flour percentage based on dry ingredients	86.16	89.77
Water (sponge and dough flour)	60.00	33.00
Total batch	176.05	144.40
Flour percentage of the total batch	56.83	69.26
Total fermentation time (sponge and dough)—hours	5 to 6	23 to 25

Conclusions

This comparative study of bread and cracker flours brings out that the cracker baker has a more difficult task in selecting suitable flour.

The difficulty arises in securing flour with bloom (related to the finished product) and natural fermentation characteristics, as in cracker baking the flour in itself is principally responsible for the desired qualities in the finished product.

A CLASSIFICATION OF BISCUIT AND CRACKER PRODUCTS FROM THE MACHINE VIEWPOINT [1]

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Before attempting to classify the different products of the biscuit and cracker manufacturer, a few words should be said about the industry itself. As this is not intended as a technical paper, a slight digression at this point may be permissible.

The writer has always considered the baking industry as consisting of three separate and very distinct branches—the large bread making concerns, the smaller hand shops, and the biscuit manufacturers. The bread baking industry is the most familiar to cereal chemists and they are largely responsible for the present high standards. The making of thousands of loaves of bread by machinery controlled by a very small group of workers requires the standardization of all factors. The cereal chemists through their researches on materials and processes have made possible this standardization.

In the small hand shop, scientific control is not vital. The small baker appreciates the excellent raw materials made possible through the strict requirements of the larger producers. At the same time, whenever he receives a shipment that requires different treatment from the previous shipment, it does not disrupt his entire routine, since each batch is an individual matter and as such receives special treatment. Baking in the smaller shops is still an art and not a science.

The biscuit and cracker industry has very little in common with the other two branches. The only real similarity between the bread industry and the biscuit industry is that in both cases the products are produced chiefly by mechanical means. Likewise it may be compared to the small hand shop only by the number and variety of the different types of goods made. Biscuits and crackers are peculiar in that they are characteristic of the Anglo-Saxon peoples. Crackers are truly American and form one of the few classes of baked products whose history may be traced back to the origin. The original cracker was pilot bread, which is

[1] Read before the Cereal Chemists Section, Pacific Intersectional Meeting, A. A. A. S. June 16, 1932.

[2] Information obtained while in the service of the Biscuit and Cracker Manufacturers' Association of America.

merely flour and water mixed together and dried in the form of thin cakes. These originated on the east coast for use as food on ships at the time when American shipping was at its pinnacle.

Biscuits are British in origin. The word biscuit, as used in this connection does not mean the hot fluffy baking powder biscuits that word conjures up for the average American, but instead the small sweet cakes ordinarily termed "cookies." To the Englishman, however, the word biscuit means a particular type of baked goods, while we use the term to designate all products that are not classed under bread, cake, or crackers.

A biscuit plant may have 70 to 80 different types of baked pieces in production. Classification is difficult unless confined to the kind of machine used to form the final product. This classification should be of especial interest to cereal chemists, as the physical characteristics of the dough or batter used for each machine must be very nearly the same for all types of pieces made by that machine, regardless of other characteristics of the finished product.

When considered from this standpoint, biscuits and crackers may be classified according to whether they are "peeled" goods or "pan" goods. "Peeled" goods are pieces that are baked directly on the shelves of the oven. A peel is a light wooden board about 22 inches wide and 40 inches long, tapering to a thin edge at one end with a short handle at the other. This is slipped under the sheet of dough as it travels on the apron from the rollers and the sheet of dough transferred in this manner to the oven shelves. All peeled goods are rolled on the same type of machine, so constructed as to give a layer of dough in the desired width and thickness and with the appropriate size and shape of the piece impressed into the dough. Included in this group are the soda crackers, such as standard sodas, saratoga flakes, family wafers, and oysters; pieces like butter thins and cream wafers and sometimes graham crackers.

Pan goods consist of all the different pieces baked on metal sheets or pans. A much greater variation is found under this heading, and instead of being made on the same machine several different types of machines are used. The various machines used for forming the biscuits may be designated as follows:

1. Cutting machine
2. Embossing machine
3. Dutch cookie machine
4. Deposit machine
5. Wire cut machine
6. Bar press machine
7. Sugar wafer machine

The cutting machine is essentially the same as that used for making crackers except that a different cutter is used, so that the cakes are cut into the desired shape. The dough is rolled into a thin layer and must be strong enough so that the scrap or unused portion of the dough can be returned automatically to the hopper. This dough is sometimes leavened, but ordinarily this machine is used where cakes of uniform size are desired.

The embossing machine is also the same machine as that used for crackers with the exception that a special cutting head is used. This head has engraved depressions which mold the biscuit into the design desired. The most familiar biscuit of this type is animal crackers. This machine is used for the different types of afternoon tea biscuits, sandwich halves, and wherever a clear-cut, finished piece is desired. The dough is never leavened and must be of such a texture as to slip easily from the embossing head without deformation, and must bake without losing the design. The English are masters in the production of this type of goods, which they call hard sweets. It is easily seen that a piece made on a machine of this kind does not allow very much latitude as regards physical texture.

The Dutch cookie machine is a self-contained unit, the main feature so far as we are concerned being the method used to form the biscuit. This machine has a large bronze roller about ten inches in diameter with cuplike depressions in the surface. These cups are engraved in any design or shape desired and the machine so constructed that the cups are filled with dough as the roller rotates. The cakes are removed from the roller by means of steel bands running in grooves flush with the bottom of the cup and over another roller. Pieces made on the Dutch cookie machine can readily be identified by the small tips attached to the pieces and by the narrow impressions formed across the face of the biscuit by the steel bands. This machine is used for pieces such as short breads containing an unusually high percentage of shortening, which is necessary in order to have the dough slip from the roller without sticking. Pieces made on the Dutch cookie machine are rarely leavened.

The deposit machine uses a dough of such a consistency that it may well be called a batter instead of a dough. This machine is constructed in such a fashion that the desired quantity of dough is ejected through nozzles by means of a pump arrangement. In order for the machine to function properly the dough must be thin enough to work through the mechanism and drop from the nozzles to the pan and at the same time it must have sufficient viscosity so that it does not spread too much on the pan and to make a fairly thick cake. Goods run on the deposit machine are invariably highly leavened and the volume of dough deposited is small compared to the volume of the baked piece. It is sometimes a

problem to obtain a fine grain in the finished piece when the dough is heavily leavened.

The wire cut machine is constructed on somewhat the same principle as the deposit machine, except that the dough used is so stiff that when forced through the nozzles it remains suspended until cut off by a small wire synchronized with the rest of the mechanism. The dough used is almost always leavened. It is almost impossible to distinguish the finished biscuit made on the wire cut machine from that made on the deposit machine.

The bar-press machine receives its name from the action on the dough. The mechanism is so arranged that the dough is pressed through dies having the shape desired in the cross section of the biscuit and falls on the apron in a long continuous bar. This bar is then cut by an auxiliary cutter into pieces of appropriate length. Examples of this type of piece are cocoanut taffy and the fig bar. This dough is rarely leavened and must be of a consistency to press through the die readily and still retain its shape during the baking process.

The sugar wafer machine is a self-contained unit used to produce the thin coverings on sugar wafers of the Nabisco type. The dough is very thin and is ordinarily referred to as batter. The machine is entirely automatic and all the operator has to do is to fill the tank with batter and remove the baked sheets. These are about 12 by 18 inches in size and are baked in an arrangement closely resembling a waffle iron.

This classification covers the various types of machines used in forming the biscuits. As previously stated, this classification is important, since it is impossible for the biscuit industry to dispense with machinery and the physical characteristics of the dough must be fixed within relatively narrow limits if the machine is to operate properly.

REGARDING THE POSSIBILITY OF CHARACTERIZING HARD AND SOFT WHEATS ACCORDING TO THE RATIO BETWEEN TOTAL AND AMINO NITROGEN OF THEIR SEEDS AND ENERGY OF AUTOLYSIS OF THE LATTER

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A number of attempts by various authors, to establish the biochemical characteristics of hard and soft wheats on the basis of total nitrogen content of the seeds, the glutenin-gliadin ratio of the gluten and of the starch content, have all been unsuccessful. While there is no doubt that marked differences exist in the morphology of various wheats, in the appearance of the seeds, and in the baking qualities of flour milled from hard and soft wheats, there is an urge to continue the search for characteristic biochemical indicators.

In 1918 one of us [1] observed that the seeds of different plants differ from each other by the relative importance of their free amino-nitrogen groups; in some of them the amount of amino-nitrogen in comparison with the total nitrogen content of the seeds is large, while in others it is small. The ratio between total nitrogen and amino-nitrogen has been called *the amino-index of the seed.* At that time determinations were made of the amino-index of the seeds of one soft and two hard wheats which gave the following results:

Kind of wheat	$\frac{\text{Total N.}}{\text{Amino N.}}$
Soft	21.3
Hard (Arnautka)	41.5
Hard (White Turkey)	48.6

Of course, all the three wheats were grown under identical conditions (on the experimental field of the Voronezh Institute of Agriculture). Each figure represents the average of several determinations. The values obtained seem to indicate that the amino-index of seeds of hard wheats is higher than that of soft wheats. In order to verify and confirm this conclusion we have at present carried out analyses on another

[1] Unpublished paper.

series of seeds also grown under identical conditions (not at Voronezh, however, but considerably farther north, on the experimental fields of the Moscow Grain Experiment Station). Exactly the same methods of analysis were used as in the previous case, viz., total nitrogen was determined by the Kjeldahl method and amino-nitrogen by the Sørensen method. The results obtained fully confirmed the previous data.

TABLE I

AMINO INDEX OF DIFFERENT VARIETIES AND CLASSES OF WHEAT

Class of wheat	Total nitrogen per gram of flour	Amino-nitrogen per gram of flour	Total N. / Amino N.
	mgms.	*mgms.*	
Soft wheats			
1. Banatka No. 3137/III	15.9	1.22	13.0
2. Banatka No. A3946	27.7	1.19	23.4
3. Banatka No. C3680	25.0	1.16	21.5
Hard Wheats			
1. White Turkey No. 5333	22.3	0.89	25.3
2. White Turkey No. 5331	21.3	0.78	27.3
3. White Turkey No. 299/III	22.0	0.67	33.0

Hard wheats of almost the same average content of total nitrogen (which is somewhat more constant in them than in soft wheats) are characterized by a considerably smaller amount of amino-nitrogen and consequently by greater amino-indexes.

In 1919 it was shown by one of us [2] that for a number of plants the energy of autolysis of ground seeds shows a definite positive correlation with the value of the amino-index. The following two sets of figures in Table II illustrate this:

TABLE II

AMINO-INDEX AND ENERGY OF AUTOLYSIS OF VARIOUS SEEDS

Name of plant	Amino-index of seed	Energy of autolysis
Mung-bean (Phaseolus aureus)	10.94	4.10
Peganum harmala	5.00	0.90
Vicia costata	8.64	3.76
Hibiscus esculentus	7.18	4.90
Hibiscus cannabinus	16.03	9.96
Oats (Avena sativa)	15.82	6.43
Wheat (soft)	21.30	12.30
Kidney-bean (Phaseolus vulgaris)	7.82	2.45
Horse-bean (Vicia faba)	12.40	7.06
Lupinus luteus	12.36	6.92
Lepidium sativum	10.20	5.69
Sinapis alba	12.70	7.93
Brassica napus var. esculenta	21.06	14.72
Lentils (Lens esculenta)	9.20	3.40

[2] Blagoveschensky, A. "Sur la peptase des graines." J. de la Soc. Bot. russe, 1919, 4: 52–77.

The coefficient of correlation between the two sets calculated according to formula $r = (\Sigma\delta x \cdot \delta y / \sqrt{\Sigma\delta x^2 \cdot \Sigma\delta y^2})$ equals $r = +0.798$, i.e., the correlation is very definite.

It seemed interesting to us to determine the energy of autolysis also for the seeds of the hard and soft wheats under investigation. For this purpose, into weighings of 1 gm. of seed flour 5 cc. of water was poured, some toluene was added and the extent of proteolytic disintegration of the protein was determined after 24 hours by formol titration by the Sørensen method. The following results (Table III) were obtained:

TABLE III

AMINO-INDEX AND ENERGY OF AUTOLYSIS VALUES OF WHEATS

Class and variety of wheat	Amino-index	Average	Energy of autolysis	Average
Soft wheats				
Banatka 3137/III	13.0	19.3	1.45	1.60
Banatka A3946	23.4		1.72	
Banatka C3580	21.5		1.62	
Hard wheats				
White Turkey 5333	25.3	28.5	2.05	2.26
White Turkey 5331	27.3		1.78	
White Turkey 299/II	33.0		2.96	

Thus the relation we found between the value of amino-index and the energy of autolysis is clearly confirmed in the present case. The higher the amino-index and the more densely the amino-acids in the protein molecule are packed, the more forceful is the disintegration of that molecule by autolysis. We will not attempt to touch here upon the question of probable importance of this phenomenon from the biological standpoint, but must emphasize that a quicker disintegration of the protein in the process of bread making might be of no small importance.

In conclusion, let us discuss the question of a possible connection between the value of amino-index of the seeds and their vitreousness. Eight samples of seed were divided by appearance into 3 groups each—vitreous, semi-vitreous, and starchy. The seeds of each group were finely ground and determinations were made of the total nitrogen content and the amount of nitrogen of free amino-groups. The latter determination was carried out, not by the Sørensen method, but by the Linderström-Lang method [3] by direct titration of the amino-groups by N/5 HCl alcohol solution in an acetone solution with benzol-azo-α-naphtyl-amine (naphtyl-red) as indicator. We assumed that the cause of vitreousness might probably lie in greater compactness of the nitrogen

[3] Linderström-Lang, K. Titrimetrische Bestimmung von Aminostickstoff. Z. Physiol. Chem. 173, 32–50, 1928.

substances and that, in consequence, the amino-index of vitreous seeds would be greater than that of starchy ones. The results of the investigation have more or less confirmed this assumption, as can be seen from the data in Table IV.

TABLE IV

AMINO-INDICES OF WHEAT OF VARIOUS DEGREES OF VITREOUSNESS

Kind of wheat	Amino-indices		
	Vitreous	Semi-vitreous	Starchy
Lutescens I	40.2	35.4	38.6
Lutescens II	63.6	62.4	62.2
Lutescens III	51.7	50.5	44.0
Milturum	44.3	45.7	43.7
Ukrainka I	45.7	44.4	34.5
Ukrainka II	43.4	42.7	42.4
Ukrainka III	73.8	60.0	55.9
Melanopus	70.4	53.7	—
Average	56.6	49.4	45.9

It is necessary to point out that the samples were of different origin (from different plots grown in the Lower Volga, West Siberia, and North Caucasus) and of different stages of ripeness. This probably is no doubt responsible for the considerable variation between different samples of one and the same variety. The averages obtained do not pretend to great accuracy by any means, but a general tendency towards the reduction of the amino-index in the direction from vitreous forms to starchy ones must be admitted.

Summary

1. All conditions of growth being identical, the seeds of hard wheats possess a higher amino-index, i.e., a higher ratio between total nitrogen and the formal titratable nitrogen of the free amino-groups (Sørensen), than the seeds of soft wheats.

2. The energy of autolysis of the seeds shows a positive high correlation ($r = +0.8$) with the amino-index and therefore it is higher in hard wheats than in soft ones.

3. In the majority of cases vitreous seeds are characterized by a higher amino-index than starchy ones. Semi-vitreous seeds occupy an intermediate position.